Research on Rare Disease Provides Insight into How Sterol Consumption Affects Blood Cholesterol Levels

Background: Cholesterol belongs to a family of molecules called sterols, which are found in the membranes of all plant and animal cells. Most people metabolize about half of the animal-derived cholesterols that they ingest but only five percent or less of the plant sterols. However, patients with the rare disorder sitosterolemia not only absorb a much larger percentage of their dietary plant sterols but also have elevated blood cholesterol levels and premature coronary heart disease. Researchers suspected that the metabolic pathways for plant and animal sterols, which have similar structures, were somehow related, but the link between plant sterols and blood cholesterol levels was unclear.

Advance: Researchers studying families of patients with sitosterolemia have identified genes for two proteins that cooperate to excrete sterols from intestinal cells. Although the proteins preferentially remove plant sterols, they also remove cholesterol. Additionally, animal studies showed that increasing sterol consumption results in a corresponding increase in the expression of genes coding for the proteins.

Implications: In addition to explaining how a defect affecting plant sterol excretion can cause an increase in blood cholesterol levels as seen in patients with sisterolemia, the results suggest that subtle defects in the structure of these proteins or in their regulation may underlie the variable responses of healthy individuals to diets high in cholesterol. Therefore, in addition to laying the groundwork for treatments that may benefit sitosterolemia patients, the new knowledge may also be used to develop interventions that control cholesterol levels in individuals who have not responded well to established therapies.

Berge KE, Tian H, Graf GA, Yu L, Grishin NV, Schultz J, Kwiterovich P, Shan B, Barnes R, and Hobbs HH: Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 290: 1771-1775, 2000.

Lee MH, Lu K, Hazard S, Yu H, Shulenin S, Hidaka H, Kojima H, Allikmets R, Sakuma N, Pegoraro R, Srivastava AK, Salen G, Dean M, and Patel SB: Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. Nature Genetics 27: 79-83, 2001.

Understanding of Cholesterol Metabolism Suggests New Line of Therapeutics

Background: Researchers studying patients with Tangier disease, a rare condition characterized by the lack of high-density lipoproteins (HDL, or "good" cholesterol), discovered that defects in a transport protein called ABCA1 are associated with reduced HDL levels. Although ABCA1 is necessary for HDL formation, it was unclear if increasing production of functional ABCA1 would be sufficient to increase the amount of circulating HDL.

Advance: Researchers showed that transgenic mice engineered to produce higher-than-normal amounts of ABCA1 had elevated HDL levels in their blood. Compared to the "control" mice, they also excreted more cholesterol in their bile.

In a separate study, researchers characterized several of the steps involved in production and recycling of ABCA1. Moreover, they demonstrated that preventing ABCA1 localization to the cell membrane impairs removal of intracellular cholesterol.

Implications: The observation that increasing production of ABCA1 increases circulating levels of HDL and excretion of cholesterol suggests a new pharmacological approach to preventing heart disease. Understanding the steps required to get ABCA1 to the cell membrane where it can facilitate cholesterol removal could lay the groundwork for the development of new drugs capable of improving cholesterol profiles and lowering cardiovascular disease risk.

Vaisman BL, Lambert G, Amar M, Joyce C, Ito T, Shamburek RD, Cain WJ, Fruchart-Najib J, Neufeld ED, Remaley AT, Brewer HB Jr, and Santamarina-Fojo S: ABCA1 overexpression leads to hyperalphalipoproteinemia and increased biliary cholesterol excretion in transgenic mice. The Journal of Clinical Investigation 108: 303-309, 2001.

Neufeld EB, Remaley AT, Demosky SJ, Stonik JA, Cooney AM, Comly M, Dwyer NK, Zhang M, Blanchette-Mackie J, Santamarina-Fojo S, and Brewer HB Jr: Cellular localization and trafficking of the human ABCA1 transporter. Journal of Biological Chemistry 276: 27584-27590, 2001.

Alzheimer's Genetic Factor is Linked to Sleep Apnea

Background: Sleep apnea, a common disorder in which people stop breathing temporarily during sleep, has been associated with potentially serious effects on cardiopulmonary health, diminished quality of life due to excessive daytime sleepiness, and social and cognitive problems in children and adults. Sleep apnea and its corresponding cardiovascular complications occur disproportionately in some families, although the basis for this phenomenon is not understood. Sleep apnea also affects many Alzheimer's disease patients.

Advance: New findings indicate that a genetic marker already linked to Alzheimer's disease, apolipoprotein E ε4 (ApoE ε4), might also be a factor influencing sleep apnea susceptibility. This marker is associated with a two-fold increased risk of sleep apnea and a 35 percent increase in severity of apnea symptoms in patients who do not have Alzheimer's disease. Association of sleep apnea with ApoE ε4 suggests that apnea may contribute to cognitive impairment in those who possess this marker and later develop Alzheimer's disease, since sleep apnea lowers blood oxygen levels during sleep. Reduced levels of blood oxygen could potentially increase the severity of brain damage caused by Alzheimer's disease.

Implications: These findings suggest that treating sleep apnea in Alzheimer's patients might improve their cognitive function and quality of life. They also indicate that it may be possible to identify people at greatest risk for sleep apnea, thereby contributing to the development of new management approaches to reduce their rate of cognitive impairment should they develop Alzheimer's disease.

Kodotani H, Kadotani T, Young T, Peppard PE, Finn L, Colrain IM, Murphy GM Jr, and Mignot E: Association between apolipoprotein E ε4 and sleep-disordered breathing in adults. The Journal of the American Medical Association 285: 2888-2890, 2001.

Genetic Mutations and Altered Proteins Influence Lymphangioleiomyomatosis

Background: Lymphangioleiomyomatosis (LAM) is a rare and devastating lung disease, primarily affecting young women, that is characterized by an overgrowth of smooth muscle-like cells in the lung. Lung function worsens steadily due to overgrowth of the LAM cells and formation of numerous cysts throughout the lungs. LAM cases appear sporadically and do not seem to be inherited. However, a link has been observed between LAM and another, more common, inherited condition known as tuberous sclerosis complex (TSC). Many women with TSC also have a mild form of LAM. Additionally, women with TSC, as well as women with LAM, often develop unusual, benign kidney tumors containing typical LAM cells.

Advance: Researchers have discovered mutations in two genes, *TSC1* and *TSC2,* that are associated with the development of LAM in women with TSC. Normally, the genes code for proteins that suppress tumor formation. Although the effects of the *TSC1* mutations on LAM development are unclear, the mutations in *TCS2* are thought to affect the cell signaling properties of the *TCS2* protein, tuberin, and its ability to regulate growth of smooth muscle cells.

Implications: There is no cure for LAM, although lung transplantation has helped some patients and anti-estrogen therapy may alleviate some of the symptoms. Understanding the influences of specific genes in LAM, as well as the roles of specific proteins, should aid in identifying new therapeutic targets and developing new treatments for this debilitating disease.

Strizheva GD, Carsillo T, Kruger WD, Sullivan EJ, Ryu JH, and Henske EP: The spectrum of mutations in *TSC1* and *TSC2* in women with tuberous sclerosis and lymphangioleiomyomatosis. American Journal of Critical Care Medicine 163: 253-258, 2001.

Link Identified Between Ethnicity and "Asthma Genes"

Background: From 1980 to 1996, the number of Americans afflicted with asthma more than doubled to almost 15 million. Although asthma affects Americans of all ages, races, and ethnic groups, minority populations experience substantially higher rates of fatalities, hospital admissions, and emergency room visits due to asthma.

Asthma appears to be influenced by genetic components. It is likely that several genes, each with relatively small effects, act together with environmental exposures to determine an individual's overall risk of developing the disease.

Advance: Three separate searches for asthma and allergy genes in members of black, white, and Hispanic ethnic groups indicate that genes on chromosomes 5q, 8p, 12q, 14q, and 15q are associated with asthma regardless of a patient's ethnicity. Depending on the patient's ethnic background, however, genes on one of three other chromosomes also are involved. For example, a gene on chromosome 1p correlates with asthma in Hispanics, but not in blacks or whites. Unlike asthma in blacks and Hispanics, asthma in whites is associated with a gene on chromosome 6p. Similarly, a gene on chromosome 11q influences the development of asthma in blacks, but not in whites and Hispanics.

Implications: Identifying genetic factors that predispose to asthma development will help to identify individuals at risk for developing asthma. Understanding the genetic variations of asthma is also likely to have a major influence on improving therapeutic options available to patients. [secondary – therapeutics]

Xu J, Meyers DA, Ober C, Blumenthal MN, Mellen B, Barnes KC, King RA, Lester LA, Howard TD, Solway J, Langefeld CD, Beaty TH, Rich SS, Bleecker ER Cox NJ, and the Collaborative Study on the Genetics of Asthma: Genomewide screen and identification of gene-gene interactions for asthma-susceptibility loci in three U.S. populations: collaborative study on the genetics of asthma. American Journal of Human Genetics 68: 1437-1446, 2001.

Studies Cast New Light on How Pneumonia Develops in AIDS Patients

Background: *Pneumocystis carinii* (*P. carinii*) is one of the most important causes of opportunistic infection in the lung, especially among individuals with an impaired immune system, such as AIDS patients and patients undergoing immunosuppressive therapy for organ transplantation or cancer. When *P. carinii* is free to grow in the lung, the usual result is pneumonia. For many years, *P. carinii* pneumonia was considered a reactivation of latent infection acquired earlier in life. Since antibodies to *P. carinii* are often found in children, indicating early exposure to the organism, it was believed that infections in childhood are never completely cured but are kept in check by the immune system. However, some doubt was cast on this dogma when a study performed in the early 1990s showed that autopsied lungs of adult individuals with normal immune systems contained no residual trace of *P. carinii*, suggesting complete eradication of the organism following childhood infection in most cases.

Advance: In two recent studies of HIV-infected patients in San Francisco and Cincinnati, researchers found clustering of cases of *P. carinii* infection in specific geographic areas. Patients living within these clusters had a significantly higher rate of *P. carinii* infection than similar patients from other areas. Surprisingly, these results could not be explained by poverty or lower socioeconomic status, since cases tended to occur in the more affluent areas. These findings complement the 1990s study and strongly suggest that infection in immune compromised patients is due to acquisition rather than reactivation.

Implications: *P. carinii*, the leading cause of severe pneumonia in AIDS patients, presents a major challenge to investigators because it is impossible to grow the organism reliably in the laboratory, thereby limiting the ability to study transmission of the organism in humans and animals. The new data support the hypothesis of a common environmental reservoir for *P. carinii* in the geographic areas analyzed and of direct transmission between individuals. More research is needed to identify its environmental reservoirs and to learn how *P. carinii* is spread, so that measures can be taken to prevent transmission among susceptible individuals. This knowledge may also lead to improvement in guidelines for how preventive therapy is administered. [secondary – prevention]

Dohn MN, White ML, Vigdorth EM, Buncher CR, Hertzberg VS, Baughman RP, Smullian AG, and Walzer PD: Geographic clustering of *Pneumocystis carinii* pneumonia in patients with HIV infection. American Journal of Respiratory and Critical Care Medicine 162: 1617-1621, 2000.

Morris AM, Swanson M, Ha H, and Huang L: Geographic distribution of human immunodeficiency virus-associated *Pneumocystis carinii* pneumonia in San Francisco. American Journal of Respiratory and Critical Care Medicine 162: 1622-1626, 2000.

Mutation is Associated with Familial Interstitial Lung Disease

Background: Interstitial lung diseases are a group of disorders characterized by thickening of the lung tissue. Although their causes are unknown, some of the disorders, including chronic pneumonitis of infancy, may have a genetic basis.

Healthy lungs require pulmonary surfactant, a mixture of lipids and proteins found on the surface of healthy lung tissue that prevents lung collapse during normal breathing. Surfactant deficiency is the principal cause of respiratory distress syndrome in premature infants and the inability to produce surfactant protein B causes fatal neonatal lung disease. Thus, researchers hypothesized that a defect in surfactant production may also contribute to the development of interstitial lung disease.

Advance: Researchers studying a mother who developed interstitial lung disease as a child but showed no respiratory symptoms at birth and her infant who also had no respiratory symptoms at birth but subsequently developed interstitial lung disease found mutations in the gene for a specific lung protein called surfactant protein C. Analysis of lung tissue from both mother and infant suggested that the protein was not being processed and secreted properly, resulting in lower than normal levels of the protein.

Implications: This research, which suggests a new etiology for interstitial lung disease, will lead investigators to examine the role of surfactant protein C in the disease process. Identifying the surfactant protein C gene mutation associated with interstitial lung disease will lead to more accurate classification of the disease in both children and adults and could eventually lead to more effective use of available therapies to treat the disease.

Nogee LM, Dunbar AE, Wert SE, Askin F, Hamvas A, and Whitsett JA: A mutation in the surfactant protein C gene associated with familial interstitial lung disease. The New England Journal of Medicine 344: 573-579, 2001.

Novel Protein Links Fanconi Anemia with Other Chromosome Instability Syndromes

Background: Fanconi anemia (FA) is a hereditary disorder characterized, in part, by a deficient DNA-repair mechanism that increases a person's risk for a variety of cancers. Although the molecular basis of FA is largely unknown, researchers have established that five different FA proteins form a multi-protein complex; mutations affecting any one of the proteins can prevent DNA repair, cause chromosome breakage and genomic instability, and affect the regulation of cell growth and death. Although researchers knew that the protein complex resides in the nucleus, no one knew how it mediated DNA repair. Moreover, many FA patients did not have mutations in known FA genes, and researchers hypothesized that they might have mutations in a gene that codes for the "missing link" between the DNA repair machinery and the known FA proteins.

Advance: Researchers identified a new protein, called FANCD2, that serves as the "missing link" between the FA protein complex and DNA-repair machinery. In response to DNA damage, the FA complex activates FANCD2, which then associates with BRCA1, a tumor-suppressor protein made by a "breast cancer susceptibility gene." Although it is unclear if FANCD2 binds directly to BRCA1 or if the interaction is mediated by another, unidentified protein, researchers suspect that other known DNA repair proteins are associated with activated FANCD2.

Implications: Because BRCA1 also associates with DNA repair proteins that are defective in other chromosome-instability syndromes, such as Bloom syndrome, ataxia telangiectasia, and Nijmegen breakage syndrome, its association with the new FA protein, FANCD2, suggests that the syndromes share a common mechanism despite their differences in chemical sensitivities and clinical abnormalities. The link between the BRCA1-mediated tumor suppression protein and the FA pathway also raises the possibility that mutations in the FA pathway are involved in the development of breast cancers in which mutations are not present in the BRCA1 gene.

Timmers C, Taniguchi T, Hejna J, Reifsteck C, Lucas L, Bruun D, Thayer M, Cox B, Olson S, D'Andrea AD, Moses R, and Grompe M: Positional cloning of a novel Fanconi anemia gene, *FANCD2*. Molecular Cell 7: 241-248, 2001.

Garcia-Higuera I, Taniguchi T, Ganesan S, Meyn MS, Timmers C, Hejna J, Grompe M, and D'Andrea AD: Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. Molecular Cell 7: 249-262, 2001.

Functional Brain Imaging as a Tool to Understand Cochlear Implant Performance

Background: The cochlear implant, an electronic device, was the first clinically useful neural sensory prosthesis to replace a human sense. This device has allowed individuals who lost their hearing as adults to recover an ability to understand speech. Although speech perception performance of adults has steadily increased with new advances in cochlear implantation, wide performance variations exist among cochlear implant recipients. Differences in structural and functional abnormalities of the auditory system may play a role in this variability. Yet, little data exists in humans regarding auditory system abnormality or reorganization following deafness, or on the preservation or recovery of auditory function following cochlear implantation.

Advance: Recently, functional neuroimaging techniques have been used to assess the brain activity associated with auditory performance. These studies have now been extended to individuals with cochlear implants. To examine the possibility that reduced auditory cortex activity might contribute to the wide range in perceptual performance found in cochlear implant users, NIH-funded investigators completed preliminary studies examining functional brain imaging using single photon emission computed tomography (SPECT) in individuals before and after cochlear implantation. The data suggest that preoperative to postoperative changes in auditory cortex responsiveness as measured by SPECT imaging are related to improvements in speech perception scores. Also, despite relatively similar hearing losses in each ear, significant differences in preoperative auditory cortex activation were observed between ears, which may help guide selection of the most appropriate ear for implantation.

Implications: These data suggest that functional brain imaging may be a useful tool for exploring the responsiveness of the auditory cortex in cochlear implant candidates and for understanding individual performance variability.

Roland S, Tobey EA, and Devous MD Sr: Preoperative functional assessment of auditory cortex in adult cochlear implant user. Laryngoscope 111: 77-83, 2001.

Exploiting Mouse Models of Disease: Discovery of Novel Deafness Genes and Genetic Interactions that Modify Hearing Impairment

Background: NIH has developed a substantial program for the study of existing mouse mutants as well as the creation of new mutant mice to facilitate the discovery and analysis of genes responsible for hereditary hearing impairment in humans.

Advance: In a recent study utilizing the mouse mutant Waltzer, NIH-supported scientists showed that mutations in members of the human cadherin gene family cause Usher Syndrome type 1D. The function of cadherins include cell and tissue polarization, cell sorting, cell migration and cell rearrangements. This study should expedite identification of the mechanisms by which cadherin mutations cause this devastating deafness and blindness syndrome.

In other studies, a mouse nuclear gene previously identified by NIH-supported researchers has now been shown to interact with mutated genes in the mitochondria to significantly alter the severity of age-related hearing loss. This model system should provide important information regarding age-related hearing loss in humans, a relatively common and debilitating health problem of the aging U.S. population.

Implications: These findings clearly illustrate the power of mouse genetics and the value of mouse models of deafness for the identification and detailed molecular characterization of human hearing impairment.

Johnson KR, Zheng QY, Bykhovskaya Y, Spirina O, and Fischel-Ghodsian N: A nuclear-mitochondrial DNA interaction affecting hearing impairment in mice. Nature Genetics 27: 191-194, 2001.

Alagramam KN, Murcia CL, Kwon HY, Pawlowski KS, Wright CG, and Woychik RP: The mouse Ames waltzer hearing-loss mutant is caused by mutation of Pcdh15, a novel protocadherin gene. Nature Genetics 27: 99-102, 2001.

Hearing Parents of Deaf Children Favor Genetic Testing for Deafness

Background: Genetic testing has become an option for deaf individuals and their families. However, little attention has been given to the value and impact of such testing, as perceived by the public. To investigate this issue, parents with normal hearing who have one or more deaf children were surveyed about their attitudes toward diagnostic, carrier, and prenatal testing for deafness. This population was chosen because over 90 percent of deaf children are born to persons with normal hearing.

Advance: The main result of the study was that 96 percent of the respondents had a positive attitude toward genetic testing for deafness, including prenatal testing. None of these parents stated that they would terminate an affected pregnancy. The most common reason given for wanting testing (93 percent) was to identify the cause of deafness. Other common reasons included determining the recurrence risk for future children and refining the affected child's future medical management and/or treatment. The results of this study contrast sharply with previous surveys of *deaf adults* (who may or may not have deaf and/or hearing children) who had a predominantly negative attitude toward genetic testing for deafness, with the majority stating that they believed such tests would do more harm than good. Importantly, questioning of the subjects revealed a lack of understanding of the implications of the information provided by genetic tests.

Implications: Genetic testing should be combined with appropriate genetic counseling, to ensure that parents and patients are given clear, helpful information that they can understand and use.

Brunger JW, Murray GS, O'Riordan M, Matthews AL, Smith RJH, and Robin NH: Parental attitudes toward genetic testing for pediatric deafness. American Journal of Human Genetics 67: 1621-1625, 2000.

An Essential Gene in Development of the Auditory and Vestibular Systems

Background: An increasing number of genes involved in inner ear development are being discovered. Mutations in a number of these genes have been shown to result in structural and functional abnormalities of the auditory and/or vestibular systems.

Advance: A team of NIH-supported investigators, at the Baylor College of Medicine and the University of Illinois at Chicago, have used a mouse model to discover the first gene shown to regulate sensory cell and neuronal development within both the auditory and vestibular systems. This gene, *BETA2/NeuroD1,* is critical for the formation of neurons in the cochlear-vestibular ganglion (CVG), the neuronal bundle carrying signals from the inner ear to the brainstem auditory and vestibular centers. At very early stages of development, absence of *BETA2/NeuroD1* retards the delamination and differentiation of the CVG neuronal precursors (neuroblasts). Later, inadequate trophic support and excess programmed cell death (apoptosis) also contribute to the reduction of CVG neurons. Lack of *BETA2/NeuroD1* also causes alterations in the sensory cells and supporting structures of the inner ear. In addition to its peripheral effects, *BETA2/NeuroD1* acts centrally in both auditory and vestibular systems. Its loss eliminates the dorsal cochlear nucleus neurons and the granule cells of the posterior cerebellum, structures critical to hearing and balance, respectively. The *BETA2/NeuroD1* null mouse is completely deaf and suffers from severe imbalance and incoordination, manifested by head tilting, inability to right themselves when laid on their sides or backs, circling behavior and ataxia.

Implications: BETA2/NeuroD1 is the first gene shown to regulate development of two functionally related structures in the nervous system involved in the control of balance, the vestibular ganglion and the posterior cerebellum. The identification of this gene and an understanding of its normal function in the control of balance will assist researchers designing therapies for balance control as well as formulating preventative measures to preserve control of balance.

Liu M, Pereira FA, Price SD, Chu MJ, Shope C, Himes D, Eatock RA, Brownell WE, Lysakowski A, and Tsai MJ: Essential role of BETA2/NeuroD1 in development of the vestibular and auditory systems. Genes and Development 14: 2839-2854, 2000.

The Genetic and Environmental Etiology of Stuttering

Background: Stuttering is a disorder in the production of fluent speech. Within the last two decades, NIH-supported scientists have shown that genetic factors may predispose individuals to stutter.

Advance: The present twin study screened a large population-based twin sample from the Australian Twin Registry. Telephone interview-based diagnoses were obtained for 457 of these individuals. Approximately 70 percent of the variance in risk for stuttering was found to be attributable to additive genetic effects, with the remainder due to unshared environmental effects (e.g. birth events, traumas or illnesses, peer influences etc). There is preliminary evidence to suggest that two subgroups of affected cases may exist: stutterers whose etiology is primarily "genetic" in origin (those with a positive family history) and non-familial (sporadic) cases whose stuttering may have been precipitated by early brain damage.

Implications: This study provides additional support for research to identify the genes whose mutation predisposes individuals to stutter in families with a history of stuttering.

Felsenfeld S, Kirk KM, Zhu G, Statham DJ, Neale MC, and Martin NG: A study of the genetic and environmental etiology of stuttering in a selected twin sample. Behavior Genetics 30: 359-366, 2000.

Identification of Genes Causing Deafness in Humans

Background: Hearing loss occurs with a frequency of about 1 in 1,000 newborns and is a prevalent, but not necessarily inevitable, feature of the aging process. There are several causes of hearing loss in youngsters and the elderly include viral and bacterial infections, loud noise, head trauma, ototoxic chemicals or the inheritance of dysfunctional genes. The underlying biological processes responsible for hearing loss are not well understood for any of these various etiologies. NIH scientists are identifying the genes that are associated with hearing loss in all age and ethnic groups.

Advance: Recently, NIH scientists reported cloning a gene for recessively inherited Usher syndrome type 1D (USH1D) located on chromosome 10. They showed that USH1D encodes a protein called cadherin-23. Mutations of this gene cause USH1D. Individuals who inherit two defective copies of this gene are born profoundly deaf and gradually lose their sight beginning in adolescence. Surprisingly, a less profound mutation of USH1D appears to cause severe deafness but does not cause the loss of sight. Studies are underway to determine the function of cadherin-23 in the ear and eye. Knowledge of the function of cadherin-23 in the eye may help us develop a method to prolong eyesight in these individuals.

Scientists also identified a gene located on chromosome 21 whose mutation caused recessively inherited hearing loss (DFNB29). This gene encodes a protein called claudin 14, which is believed to help seal cells together in the ear thus preventing the leakage between cells of a fluid (endolymph) which bathes the cells that convert sound into an electrical signal that is sent to the brain. Studies are underway to determine the function of claudin 14 in a mouse model.

Implications: Identification of these genes, as well as the understanding of their normal functions in the ear and eye are entry points that will allow researchers to think about and begin designing therapies for hearing loss as well as to formulate preventative measures to preserve hearing and sight.

Bork JM, Peters LM, Riazuddin S, Bernstein SL, Ahmed ZM, Ness SL, Polomeno R, Ramesh A, Schloss M, Srisailpathy CRS, Wayne S, Bellman S, Desmukh D, Ahmed Z, Khan SN, Der Kaloustian VM, Li XC, Lalwani A, Riazuddin S, Bitner-Glindzicz M, Nance WE, Liu XZ, Wistow G, Smith RJH, Griffith AJ, Wilcox ER, Friedman TB, and Morell RJ: Usher syndrome ID and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel cadherin-like gene *CDH23*. American Journal of Human Genetics 68: 26-37, 2000.

Wilcox ER, Burton QL, Naz S, Riazuddin S, Smith TN, Ploplis B, Belyantseva I, Ben-Yosef T, Liburd NA, Morell RJ, Kachar B, Wu DK, Griffith AJ, Riazuddin S, and Friedman TB: Mutations in the gene encoding tight junction claudin-14 cause autosomal recessive deafness *DFNB29*. Cell 104: 1-20, 2001.

Ahmed ZM, riazuddin S, Bernstein SL, Ahmed Z, Khan S, Griffith AJ, Morell RJ, Friedman TB, Riazuddin S, and Wilcox ER: Mutations of the protocadherin gene *PCDH15* cause Usher syndrome type 1F. American Journal of Human Genetics 69: 25-34, 2001.

Disability Continues to Decline for Older Americans

Background: Results of surveys conducted through the early 1990s suggested that the prevalence of chronic disability in the older U.S. population was lower than had been predicted. It was not known, however, exactly how much the rate had declined and if it was expected to continue. Researchers analyzed data from the 1982, 1989, 1994, and 1999 waves of the National Long Term Care Survey (NLTCS) to determine the size of the reduction from 1982 through 1999, and to determine if the rate of decline had increased in the most recent survey period. The researchers also examined trends in disability rates among elderly black Americans in comparison to those observed in non-black populations.

Advance: The 1999 NLTCS continues to document a dramatic decline in the overall prevalence of disability among older Americans over the past two decades. The number of chronically disabled persons in 1999 was 7.0 million – 2.3 million fewer than would have been disabled if rates had not changed between 1982 and 1999. The improvements in recent years are also noteworthy for a newly observed decrease of at least 200,000 in the number of people estimated to live in nursing homes. An important aspect of the chronic disability patterns is the sharp reduction in disability rates among black Americans during the 1990s. From 1994 to 1999 the estimated decline (5.9 percent) in disability prevalence was even larger for black Americans than for 1989 to 1994.

Implications: The findings from the NLTCS show that the decline in disability rates among older Americans has accelerated in recent years, and includes a more diverse group of Americans. This research could have important policy implications for planning for health care utilization, Medicare, Social Security, and long-term care. In addition, decreased rates of disability could mean that more individuals could remain in the work force for longer periods of time, rather than being forced into retirement by poor health. Further research is needed to determine the factors that contribute to the declining disability rates in order to maintain or even accelerate this decline and thus improve the health and function of more older Americans.

Manton KG, and XiLiang G: Changes in the prevalence of chronic disability in the United States black and nonblack population above age 65 from 1982 to 1999. Proceedings of the National Academy of Sciences USA 98: 6354-6359, 2001.

Increase in Maximum Age at Death

Background: A fundamental question in aging research is whether humans and other species possess a definite lifespan limit. National demographic statistics suggest that the maximum age at death has been rising steadily in industrialized countries for more than 100 years. Two important questions arise from this observation. First, has this upward trend been steady over time, or has it changed pace in recent years? Second, what accounts for the increase in the maximum age at death? Swedish national demographic data from 1861 to 1999 represent the longest available series of reliable information on the upper limits of achieved human lifespan. No other country's data offer the possibility for reliable trend analysis in extreme old age over such a long period.

Advance: Researchers examined the maximum age at death in Sweden, which rose from about 101 years during the 1860s to about 108 years during the 1990s. The pace of increase was steady prior to 1969 but accelerated after that date. More than 70 percent of the rise in the maximum age at death from 1861 to 1999 is attributable to improvements in the survival of individuals above age 70. The rest of the increase in lifespan is due to larger birth cohorts and increased survivorship from infancy to age 70, which increase the probability that at least one individual will survive to an extreme old age. Continuing research on this topic seeks to uncover the relationship between the increase of maximum age at death in Sweden and the comparable trend for the entire human population.

Implications: Since mortality histories for the countries of Western Europe and North America are largely similar to the Swedish experience, the results from Swedish data should apply broadly to the populations of other highly industrialized countries. This analysis suggests that human life span may not be fixed and unchanging over time. It is not known whether declines in death rates at older ages will continue to gradually extend the limits of achieved human longevity even further. Increases in lifespan and numbers of individuals surviving to extreme old age have implications for the need for health care and other services in the future.

Wilmoth JR, Deegan LJ, Lundström H, and Horiuchi S: Increase of maximum life-span in Sweden, 1861-1999. Science 289: 2366-2368, 2000.

Persistence of Cognitive Decline After Coronary-artery Bypass Surgery

Background: With advances in surgical techniques and anesthesia, elderly individuals now routinely undergo surgical procedures late in life without serious mortality risks. One of the more common types of surgeries performed in the elderly is coronary artery bypass grafting (CABG). It is known that CABG may have adverse effects on the brain including stroke, post-operative delirium, short-term cognitive impairment, and possible long-term cognitive deterioration. While improved anesthetic and surgical techniques have reduced mortality, there has been little, if any, improvement in cognitive problems following this procedure.

Advance: Longitudinal assessment of older individuals undergoing CABG surgery revealed that cognitive function at discharge predicted long-term cognitive function. NIH-supported investigators reported a high incidence of cognitive decline at discharge (53 percent) in a group of 261 CABG patients with a mean age of 61 years. Cognitive decline was defined as a reduction of more than approximately 20 percent from the patient's baseline performance on five tests of memory and related functions. The patients, as a group, went on to show a pattern of early improvement at 6 weeks (36 percent) and 6 months (24 percent), thus bolstering previous beliefs that the cognitive decline is transient. At the 5-year assessment, however, 42 percent of the surgery group was performing below baseline cognitive levels. Additional predictors of later decline included older age at surgery and lower level of education.

Implications: The finding that early cognitive improvements at 6 weeks and 6 months is not sustained over longer periods of time in a large proportion of CABG patients is of great interest and importance. Perioperative injury, increased susceptibility to injury, or decreased ability to recover from injury, may be responsible for cognitive dysfunction after CABG surgery and will be important research issues to pursue. The effect of other factors impacting on the extent of cognitive decline, including ongoing coronary or cerebrovascular disease, will be additional areas of future research.

Newman MF, Kirchner JL, Phillips-Bute B, Gaver V, Grocott H, Jones RH, Mark DB, Reves JG, and Blumenthal JA: Longitudinal assessment of neurocognitive function after coronary-artery bypass surgery. The New England Journal of Medicine 344: 395-402, 2001.

More African-Americans than Africans get Alzheimer's Disease

Background: If populations can be identified that have significantly lower or higher incidence of Alzheimer's disease (AD), this will greatly facilitate the search for both genetic and non-genetic risk factors for the disease.

Advance: Over a five-year period, researchers followed 2,147 African-Americans in Indianapolis, Indiana and 2,459 Yoruba in Ibadan, Nigeria, age 65 and older, to see if they developed dementia and AD. The screening instrument that was used, the Community Screening Interview for Dementia, was developed specifically for use in comparative epidemiological studies of dementia in culturally disparate, non-literate, and literate populations. All clinically assessed participants at both sites received the same examination with included a structured interview with an informant, neuropsychological testing, examination by a physician, and laboratory and imaging studies, when deemed clinically appropriate. Great care was taken to ensure that diagnostic consistency was maintained within and between sites. The results indicated that in the African-American group, 3.24 percent per year developed dementia, including 2.52 percent per year who developed AD. In the African group, 1.35 percent per year developed dementia, including 1.15 percent per year who developed AD. The majority of those who developed a dementing disorder, in either country, developed AD. In both communities, two-thirds of the study subjects were female.

Implications: This is the first report of incidence rate differences for dementia and AD in studies of two populations from non-industrialized and industrialized countries using identical methods of evaluation and the same group of investigators in both sites. Further studies of these two populations will focus on identifying genetic or potentially modifiable non-genetic factors such as heart disease, diabetes, high cholesterol, and lifestyle and environmental factors. For example, there is a much lower prevalence of factors associated with vascular disease in the Yoruba as compared with the African-Americans, such as high cholesterol levels, high body mass index, hypertension, and diabetes. These lower rates of risk factors for vascular disease, in addition to or in combination with the lower strength of association of the APOE ε4 genetic risk factor for AD in Yoruba, may account for the reported difference in AD.

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Regeneration and Tissue Repair: Tapping the Potential of Stem Cells

Background: Humans are able to live a relatively long time because of processes that mend damaged muscle, repair broken bones, renew wounded skin, replenish blood and restructure vessels. Despite the inevitable physiological changes that people experience as they age, most human tissues are remarkably resistant to the insults of time because they are able to repair themselves. Exceptions are heart and nervous tissue. It has been assumed that they have little, if any, ability to replace cells severely damaged or lost during a heart attack or stroke. An unfulfilled hope is that strategies can be found to replace cells in these tissues and restore lost function.

Advances: A recent discovery suggests the human heart may be able to regenerate to some extent. When scientists examined the hearts of patients who died between 4 and 12 days after a heart attack, they found almost 100 times more cells undergoing cell division in hearts from heart attack victims than in hearts from patients who died from other causes. About 4 percent of the cells in the region damaged in the heart attack appeared to be in the process of dividing, so it appears that heart muscle has some capacity to regenerate and replace damaged tissue. While this amount of cell replacement may not be able to restore function to a badly damaged heart, it may be enough to repair damage resulting from the blockage of small capillaries.

In the mouse, adult stem cells show potential to replace cells lost in either the heart or brain. When adult bone marrow cells are injected into the mouse circulatory system, they can find their way to the damaged brain and gradually change into neuronal cells. When bone marrow cells are transplanted into mouse hearts damaged by a "heart attack," these cells regenerate not only new heart muscle but also blood vessel components. In mice, this repair can be accomplished in just a few weeks. Clearly, adult stem cells show great potential for cell replacement therapy to rebuild damaged hearts.

Implications: These reports suggest that repair of organs like heart and brain may be more possible than previously assumed. Heart cells seem able to respond to injury by dividing and forming replacement heart cells. Adult mouse bone marrow-derived cells reveal an unexpected degree of plasticity, sparking considerable optimism that age-related conditions such as heart attacks, strokes and neurodegenerative diseases can be treated by using bone marrow cells derived from patients' own bones. Similar interventions may be useful in other age-related pathologies characterized by loss of cells.

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Orlic D, Kajstura J, Chimenti S, Jakonluk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A and Anversa P: Bone marrow cells regenerate infarcted myocardium. Nature 410: 701-705, 2001.

Growth Hormone Deficiency Promotes Longevity in Mice

Background: Production of growth hormone gradually decreases with age, leading some scientists to propose that regular injections of growth hormone would reverse or delay at least some adverse age-related changes in body composition. In a study a decade ago, scientists showed that weekly growth hormone injections increased lean body mass and decreased fat tissue mass. Based on this evidence, a growing cadre of doctors promoting "anti-aging medicine" have been recommending growth hormone injections for their elderly patients.

Advance: Experiments in mice are challenging the concept that long-term growth hormone injections would reverse aging in humans. Mice with a mutation that leaves them unable to produce growth hormone live about 30-40 percent longer than normal mice. These mice have elevated levels of several anti-oxidant enzymes, which protect against tissue damage characteristic of aging. They also have reduced blood glucose levels, and increased sensitivity to insulin. These mice are also smaller than normal, and are referred to as "dwarf" mice. Although these mice are also deficient in other hormones, experiments strongly suggest that the growth hormone deficiency alone is responsible for the delayed aging observed in these mice.

Not only is lifespan extended by the growth hormone deficiency, but mutant animals also show delays in age-dependent collagen cross-linking, a sign of tissue damage, and several age-sensitive measures of immune system status. These findings demonstrate that a single gene can regulate life expectancy and the timing of both cellular and extracellular senescence in a mammal.

Implications: These data provide new insights into hormonal regulation of senescence, longevity, and age-related disease. They also strongly suggest the need to examine more closely the effects of long term injection of growth hormone in humans before it can be considered a safe and desirable intervention to slow down or reverse aging in humans.

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Flurkey K, Papaconstantinou J, Miller RA, and Harrison DE: Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. Proceedings of the National Academy of Sciences USA 98: 6736-6741, 2001.

Structure-function Studies of the Insulin Receptor

Background: Insulin is a hormone that acts on target tissues (such as muscle and the brain), for increase glucose uptake and energy storage. Insulin acts by binding to and activating its cell surface receptor. Understanding how the insulin receptor works is important to understanding the causes of diabetes mellitus type 2, a disease that often results from defects in the receptor's activity, making cells resistant to the action of insulin (insulin resistance). The region of the insulin receptor within the cell contains structural features that may contribute to the varied actions of insulin.

Advance: To study the function of the receptor region close to the cell membrane, a shortened version of the gene that codes for this region was made and introduced into cells. This shortened gene version acts to stimulate the synthesis of this part of the receptor and increase insulin receptor activation. The protein fragment produced by the shortened gene also plays a role in regulating the cell proliferation and metabolic events in response to insulin. These findings reveal a novel regulatory mechanism that is important to understanding the early steps of insulin action. Another aspect of insulin receptor role involves the function of the thiol (sulfur-containing) chemical group in the receptor. Scientists showed that a specific cysteine, one of the amino acid building blocks of the insulin receptor, contains the only thiol chemical group within the internal region of the receptor that can react with a neighboring protein known as TRAP (thiol-reactive membrane-associated protein). This protein may regulate the function of the insulin receptor, although its effect in insulin signaling remains to be elucidated.

Implications: Type 2 diabetes is the most common form of diabetes. Yet, little is known of the metabolic factors contributing to the impairment of insulin function in this disease. The changes that occur in type 2 diabetes impair the function of the insulin receptor. This work clarifies several features of insulin receptor function, focusing on the intracellular region, and should provide several novel approaches to the development of treatments for type 2 diabetes.

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Genetic Alterations Can Cause Increased Life Span in Fruit Flies

Background: Caloric restriction is the only dietary intervention currently known to reliably increase the lifespan of rodents such as rats and mice. A calorically restricted diet is one that provides all essential nutrients, *e.g*. vitamins, minerals, essential amino acids, but provides about 30 percent fewer calories than an animal would ordinarily consume. Gerontologists have not yet discovered how caloric restriction works to both extend lifespan and delay the onset of age-related disease. Even if we did understand this, it is unlikely that many people would routinely practice caloric restriction, even to achieve comparable benefits.

Advance: Several recent studies using genetically altered fruit flies have shed some light on how caloric restriction may work. In a fruit fly mutant with an extended lifespan, researchers discovered that the mutated gene codes for an enzyme that aids the transport of dicarboxylic acids into mitochondria. These acids are involved in the mitochondrial metabolic pathways that generate energy in the form of ATP as well as detrimental reactive oxygen species. The mutation lowers the activity of the transport enzyme, allowing fewer dicarboxylic acids access to the mitochondria. This mutation may be creating a metabolic state similar to that induced by caloric restriction.

A different genetic alteration that increases fruit fly longevity involves mutation in the "insulin-like receptor" gene. This mutation reduces the activity of the insulin-signaling pathway and mimics the effect seen by similar mutations in nematodes and mice, which is to substantially increase lifespan.

Implications: It seems likely that both of these genetic mutations may lead to changes similar to those induced by caloric restriction in mice. If so, this could at least partially explain how caloric restriction works. The finding that reduced activity of the insulin-like signaling pathway extends lifespan in three different kinds of animals (fruit flies, nematodes and mice), suggests there is a direct link between insulin signaling and regulation of lifespan in animals. These results could provide the basis for developing interventions that might provide the same benefits in humans as caloric restriction does in rodents.

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Gene Required for Full Reproductive Lifespan in Women

Background: A female infant is born with all the ovarian follicles (eggs) that she will have for her lifetime. These follicles are progressively used up, particularly during ovulation after puberty, until the number falls below a threshold value, and menopause ensues. The average age of menopause is 51 years in all societies and eras where data are available, but 1-3 percent of women have premature ovarian failure (POF), going through menopause before age 40. In a number of these cases, a mutant gene is likely to be the cause, but no gene directly involved in regulating the time of menopause in women has been identified until now.

Advance: Mutations in a region of human chromosome 3 have been associated with both a condition involving drooping eyelids, blepharophimosis/ptosis/epicanthus inversus syndrome (BPES), and with POF. The gene that is mutated in these conditions, "FOXL2," has been isolated. It functions only in the eyelid and ovary, the tissues affected in BPES. FOXL2 is required to turn on the expression of a number of other genes in those tissues. When the function of FOXL2 is reduced by a mutation, the control of eyelid growth is lost, and the number of follicles in the ovary falls to a level too low to sustain a full reproductive lifespan.

Implications: These findings reveal the first gene that is critically involved in determining the number of follicles in a woman's ovary. For women with BPES, direct tests will now be available to determine whether they are likely to have POF, and therefore might choose to preserve childbearing capacity by storing follicles in early adulthood. Further work should determine whether FOXL2 acts as a "rheostat" for follicle number by determining their rate of synthesis or by reducing the loss of oocytes or follicles. Control of follicle loss, for example by increasing the activity of FOXL2, could lead to interventions that prevent or alleviate POF.

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BACE1 is the Major Beta-Secretase for Generation of Amyloid-beta Peptides in Mouse Brain

Background: Alzheimer's disease (AD) is caused by a complex cascade of events taking place over many years inside the brain. AD is characterized by deposition of amyloid-□ (A□) peptides in plaques and by the presence of neurofibrillary tangles in neurons of brain regions involved in memory and other higher cognitive functions. Many scientists believe that aggregation of A□ peptides starts the degeneration. One major focus of study has been the process by which the amyloid precursor protein (APP) is clipped apart by enzymes to release A□ fragments. One of the recently discovered enzymes that help clip A□ out of the APP protein was given the name, □-secretase. There are 2 forms of the enzyme, called BACE1 and BACE2. This study was designed to determine which of these is responsible for the production of Aβ in brain. Identification of the enzyme will help in design of drugs to inhibit □-secretase activity, in hope of slowing plaque production.

Advance: In order to understand the relative importance in the brain of the two β secretases, BACE1 and BACE2, a "knockout" mouse was developed in which the gene for the BACE1 enzyme was selectively eliminated to see whether removing it would interfere with the clipping of APP to produce amyloid. Indeed, Aβ peptides were no longer produced in brain cell cultures made from the knockout mice, showing that BACE1, rather than BACE 2, was responsible for the cleavage of APP into Aβ in the mouse brain.

Implications: Most scientists working on AD believe that Aβ plays a central, and probably causal, role in its development and that interfering with the deposition of Aβ may slow down or even prevent AD. Because they potentially play key roles in the processing of APP and the resultant deposition of Aβ, both BACE1 and BACE2 are therapeutic targets for the development of drugs to inhibit their action. The finding that BACE1 is the principal enzyme in brain will focus drug design on compounds specifically inhibiting this enzyme. Because the mice in which the BACE1 gene has been eliminated seem to develop normally, it may be possible to develop BACE1 inhibitors that interfere with Aβ deposition without negative effects on other metabolic pathways in brain or other tissues.

Cai H, Wang Y, McCarthy D, Wen H, Borchelt DR, Price DL, and Wong PC: BACE1 is the major beta-secretase for generation of Abeta peptides by neurons. Nature Neuroscience 4: 233-234, 2001.

Molecular Modulators of Memory

Background: Studies of the molecular mechanisms of learning and memory in animals identified factors that either encourage or inhibit communication among brain cells, affecting the mechanism for converting short-term to long-term memories. It is as important to inhibit irrelevant information from becoming a permanent part of memory, as it is to store important details in long-term memory. For example, memory in animals can either be enhanced or suppressed by changing the balance between factors that activate or repress specific brain proteins. Recent studies point to the brain proteins Ca^{2+}/calmodulin-dependent calcineurin and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) as critical factors for learning and memory.

Advance: Two research groups investigated how long-term changes in the way brain cells communicate with each other can result in the conversion of short-term memories into permanent ones. The studies focused on the regulation of memory storage by two of the molecular switches in the brain, calcineurin and CaMKII. Using genetic technology to turn the expression of calcineurin on and off in the mouse brain, they found that reducing the level of calcineurin enhanced learning and strengthened both short- and long-term memory. The improvements were reversed fully by turning calcineurin expression on again. Calcineurin therefore appears to constrain memory, and inhibition of calcineurin leads to improved learning and memory storage. In another study, genetically normal mice were compared to mice that had decreased levels of the protein CAMKII in the cortex of the brain. The genetically normal mice easily remembered what they had learned, while the mice that had decreased levels of CAMKII in the cortex did not. This reduction, but not elimination, of CAMKII produced mice that learned normally but remembered poorly.

Implications: These findings are a first step in understanding the molecular and cellular mechanisms underlying the establishment of permanent memories in the brain and clarify the roles of hippocampus and cortex in memory formation and storage. Newly acquired information is funneled through the hippocampus; over time the hippocampus "replays" stored information and transmits it to the cortex. Thus the cortex is capable of lasting memory storage only as a result of this repeated replaying of information by the hippocampus. These observations in adult animals have important implications for the changes in learning and memory that are seen with normal aging. Future investigations of potential shifts in the balance of these molecular factors with age, may point to therapeutic strategies for individuals with age-related cognitive decline.

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Frankland PW, O'Brien C, Ohno M, Kirkwood A and Silva AJ: _-CAMKII-dependent plasticity in the cortex is required for permanent memory. Nature 411: 309-313, 2001.

Characteristics of Adult Neural Stem Cells

Background: Neurogenesis, or the generation of new neurons, occurs throughout adulthood in specific regions of the brain. These regions contain neural stem cells that can divide and migrate to other areas of the brain. The specific kind of brain cell generated depends on the environment of that stem cell, including the presence of specific growth factors. Neurogenesis can be stimulated by exercise, brain injury, dietary modifications, and the mental activity that occurs in learning. Social stress and aging are associated with an impaired ability to make new neurons. Three recent studies have further characterized neural stem cells in adult brain.

Advance: In the first study, neural stem cells were isolated from the adult rat spinal cord and grown in culture with a growth factor called FGF-2. They formed both neurons and glial cells. When these spinal cord derived cells were transplanted to adult rat spinal cord, they formed glial cells only. Transplantation of the same cells to the hippocampus in the brain resulted in the generation of neurons in one area, the dentate gyrus region, while only glial cells were formed in other hippocampal regions. Thus, environmental cues, which vary among brain subregions, may determine the fate of a stem cell. The second report has identified a protein, called cystatin C, which works with FGF-2 to stimulate growth of neural stem cells. Interestingly, cystatin C is made by hippocampal stem cells themselves, and both FGF-2 and cystatin C stimulated neurogenesis in the adult hippocampus. Thus, neurogenesis may require the cooperation of multiple protein factors. In a third study, researchers successfully isolated neural stem-like cells from post-mortem brain tissue of newborn and adult humans. The survival and growth of these cells in culture was facilitated by the inclusion of FGF-2 and cystatin C. Similar to other stem cells, these cells could form neurons.

Implications: These studies continue to show the potential of adult derived neural stem cells to make different kinds of brain cells. Factors, such as cystatin C, may be critical in aiding the formation of new neurons in different brain regions and in the aging brain. It is unknown whether cystatin C levels decline in aging and, if so, whether this may be responsible for the low level of neurogenesis seen in aging. No doubt additional factors will be identified and exploited for their potential to enhance cell repair and replacement in the aged, injured, or diseased brain. Isolation of stem cells from adult, or even from post-mortem tissue, may provide alternatives to fetal or embryonic sources, but a careful evaluation of the properties of stem cells from all sources is needed before they are used for cell replacement therapy.

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Shihabuddin LS, Horner PJ, Ray J, and Gage FH: Adult spinal cord stem cells generate neurons after transplantation in the adult dentate gyrus. Journal of Neuroscience 20: 8727-8735, 2000.

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Designer Mice Eat More, Weigh Less

Background: "Eat more, weigh less" – it sounds like the advertising slogan of a weight loss program. But it became reality this year for a certain type of genetically engineered mouse, providing tantalizing possibilities for treating obese humans. Obesity is responsible for the deaths of 280,000 adult Americans each year, making it a leading cause of preventable deaths in the U.S[1]. The total cost of treating overweight and obese individuals approaches $100 billion annually[2]. Excess body weight and obesity also increase the risk of a range of diseases, including diabetes, heart disease, stroke, and various cancers.

Advance: For more than 10 years, a team of biochemists have studied an enzyme called acetyl-CoA carboxylase 2, or ACC2, which governs the body's ability to burn fat. This year, they discovered that mice designed to lack this enzyme eat 20 to 30 percent more food, and yet accumulate less fat and weigh about 10 percent less than normal mice. The engineered mice are otherwise normal, living and breeding well, and they've lived such lives for 2 years now, which is the average life expectancy for lab mice. Detailed biochemical studies show that the designer mice simply burn more fat than their normal counterparts.

Implications: If these results in mice hold true for humans, then a drug that blocks the function of ACC2 might allow people to lose weight while maintaining a normal diet. The study, which grew out of a desire to determine the different roles of ACC2 and its relative, ACC1, also sheds light on the normal pathways used to metabolize fat.

Abu-Elheiga L, Matzuk MM, Abo-Hashema KAH, Wakil SJ: Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2. Science 291: 2613-2616, 2001.

[1] All statistics are from *Statistics Related to Overweight and Obesity*, National Institute of Diabetes and Digestive and Kidney Diseases, NIH Publication No. 96-4158, July 1996.
[2] ibid.

Only Two Genes Needed to Form Fish Heads

Background: One of the most intriguing questions in human biology is how a single, fertilized egg can develop into a healthy baby. What tells some cells to form a liver, others to form bones, and still others to become blood vessels or nerves? And, even more basic, how does the developing embryo know right from left, up from down, and front from back so that all the organs are positioned properly? Like many interesting biological questions, these are far too complex – and unethical – to answer by studying developing human embryos. So many researchers turn to simpler organisms that are genetically similar to humans. Zebrafish eggs are ideal for these studies because they are transparent, inexpensive, and hatch only 3 days after fertilization.

Advance: A team of geneticists discovered, to their surprise, that just two genes make the difference between a normal and a headless zebrafish embryo. The genes, called *bozozok* and *chordino*, were known to be involved in early development. When the scientists "knocked out" these two genes, the resulting embryos developed into overgrown tails – entirely devoid of heads or trunks. The work further showed that the role of these two genes is to suppress the activity of a protein called BMP, which is required for tail formation but blocks head formation.

Implications: The study reveals a stunningly simple genetic mechanism, involving just three factors (the two genes and BMP), that is key to the complex development of a single egg into a fully developed fish. Because zebrafish have biochemical and genetic pathways similar to other vertebrates, the work will greatly advance our understanding of development in humans and other organisms. It will also shed light on the genetic basis of some serious birth defects.

Gonzalez E, Fekany-Lee K, Carmany-Rampey A, Erter C, Topczewski J, Wright CVE, Solnica-Krezel L: Head and trunk in zebrafish arise via coinhibition of BMP signaling by *bozozok* and *chordino*. Genes & Development 14: 3087-3092, 2000.

Scientists Identify New Chink in a Parasite's Armor

Background: Human African trypanosomiasis, known as sleeping sickness, is caused by infection with the parasite *Trypanosoma brucei*. Tsetse fly bites transmit this parasite to humans and livestock, where it feeds on blood. In an infected person, the parasite spreads throughout the entire body, causing at first high fever, weakness, headaches, joint pain, and itching. Over time, symptoms become more severe. Ultimately, people with sleeping sickness are exhausted from periods of sleep-like unconsciousness that are followed by coma and death. Although the incidence of sleeping sickness had declined dramatically by the mid-1900s, relaxed screening and surveillance have led to new epidemics of the disease over the last 30 years. *T. brucei*'s complicated life-cycle, like that of many parasites, has limited the success of both prevention and treatment.

Advance: Molecular biology researchers have concentrated efforts in studying the metabolism of trypanosomes, in part because these parasites display an unusual form of processing of the genetic material RNA, a cousin of DNA. Recently, scientists discovered a new chink in the armor of the parasite *T. brucei*. Researchers already knew that enigmatic RNA processing events called "RNA editing" took place in both the life-cycle form of *T. brucei* living in tsetse flies and in the bloodstream form of this parasite. Now, basic scientists have determined that RNA editing is an essential process for survival of the bloodstream parasite. In the course of their studies, the researchers also found a way to thwart the growth of the bloodstream form of *T. brucei* by knocking out one of the enzymes that performs the RNA editing and that is critical to its metabolism.

Implications: The new findings point to a potential new way to treat sleeping sickness by targeting the essential RNA editing function of the bloodstream form of *T. brucei*. By demonstrating that the enzyme that carries out the crucial RNA editing function is absolutely necessary for the survival of the bloodstream form of the parasite, the new work holds promise for enabling scientists to design targeted medicines to kill the parasite.

Schnaufer A, Panigrahi AK, Panicucci B, Igo RP Jr, Salavati R, and Stuart K: An RNA ligase essential for RNA editing and survival of the bloodstream form of *Trypanosoma brucei*. Science 291: 2159-2162, 2001.

Hepatitis C Study May Lead to New Treatments

Background: Every year, hepatitis C kills up to10,000 Americans and tallies a bill of $600 million in health care costs and lost wages.[1] Most of those infected develop chronic liver disease, cirrhosis, or liver cancer. Treatments for the disease usually fail, which is why finding new ways to target the virus is so important.

Advance: Structural biologists recently showed how the hepatitis C virus hijacks a host cell's protein-making machine, known as the ribosome, and forces it to churn out viral proteins for constructing more virus particles. This eventually kills the host cell and facilitates the spread of the new virus particles to other cells. Using the cryo-electron microscopy technique pioneered by the team's leader, the scientists captured the first image of viral genetic material bound to a ribosome and poised to trigger protein synthesis. The image revealed that the end of the virus' genetic material twists into a hook that snags the host's ribosomes. This viral hook forces the ribosomes to change shape dramatically, ensuring that they crank out viral proteins rather than host cell proteins.

Implications: By providing a clear view of how hepatitis C enslaves cell machinery in an infected host, the work may point to new molecular targets for drugs to treat the disease. Other viruses – such as those that cause polio, foot-and-mouth disease, and a type of herpes – are thought to use similar infection strategies. A better understanding of how hepatitis C infects cells may also advance efforts to design drugs to treat these virus infections. More generally, the work improves our understanding of how proteins are made – a cellular process essential for all life.

Spahn CMT, Keift JS, Grassucci RA, Penczek PA, Zhou K, Doudna JA, and Frank J: Hepatitis C virus IRES RNA-induced changes in the conformation of the 40S ribosomal subunit. Science 291: 1959-1962, 2001.

[1] *Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic disease.* MMWR 1998;47(No. RR-19): p. 1-33, Centers for Disease Control and Prevention.

Genetics of Cancer: Genetic Differences May Reduce Cancer Risk

Background: Tumor suppressor genes, which code for proteins that inhibit uncontrolled cell proliferation, are frequently mutated in human cancer. Cells that have a mixed pair, one normal and one mutated, of a particular tumor suppressor gene will not undergo abnormal cell proliferation leading to tumor formation. However, if the normal copy of the gene is lost, the cell becomes predisposed to abnormal growth, and if enough mutations accumulate, a tumor may form. Losing the gene is known as loss of heterozygosity (LOH), the mechanisms of which are not fully understood.

Advance: LOH can occur during cell division when chromosomes exchange DNA; this process is also known as mitotic recombination. The mechanism of mitotic recombination is not well understood; however, this team of researchers has demonstrated that the level of mitotic recombination in mice is affected by the degree of similarity between the two sets of chromosomes, one of which is inherited from each parent. When two unrelated strains of mice were bred together, the mitotic recombination and the subsequent loss of heterozygosity were diminished in their offspring. However, when subsequent breedings of the offspring were performed, resulting in a higher degree of chromosomal similarity in the third generation, the higher level of mitotic recombination and associated chromosomal exchange were restored, making these animals more susceptible to developing genetic diseases.

Implications: This finding may have implications in the development of human cancer. Genetic diversity between paired chromosomes is probably sufficient to reduce the level of mitotic recombination and therefore, reduce the risk of cancer. These data suggest, however, that if a high degree of genetic similarity exists between parents, their offspring may be more susceptible than the offspring of more genetically divergent parents to diseases, like cancer, that are associated with mitotic recombination and loss of heterozygosity.

Shao C, Stambrook PJ, and Tischfield JA: Mitotic recombination is suppressed by chromosomal divergence in hybrids of distantly related mouse strains. Nature Genetics 28(2): 169-172, 2001.

Benefits of DNA Replication Errors: How the Immune System Recognizes Invaders

Background: Development of a normal human immune system that is capable of reacting with a vast array of foreign antigens requires that the body produce a correspondingly wide variety of immune system proteins called immunoglobulins. In order to accomplish this task, the genes which code for immunoglobulin proteins undergo mutation in specific "hotspots" at rates that may be a billion-fold higher than the average mutation rate. The enzymological basis for this "hypermutation" process has been sought by immunologists for over 25 years.

Advance: NIH scientists have just reported that a recently discovered human DNA polymerase, polymerase eta, generates errors during DNA synthesis whose type and location match those arising during somatic hypermutation. This suggests that this enzyme may contribute to the function of the normal human immune system.

Implications: The hypothesis that immune system hypermutation may be the result of error-prone DNA polymerization supports the candidacy for this role of DNA polymerase eta, which is extraordinarily inaccurate in comparison with other DNA polymerases (it has an average base-substitution error rate that is at least 1000-fold higher than the other polymerases that do most of the DNA replication work.) Understanding how the immune system develops its full potential in order to recognize the wide array of substances presented to it may provide insights that allow us to bolster normal immune function and control abnormal function.

Rogozin IB, Pavlov YI, Bebenek K, Matsuda T, and Kunkel TA: Correlation between hot spots for somatic mutation in immunoglobulin genes and DNA synthesis errors by DNA polymerase □. Nature Immunology 2: 530-536, 2001.

Combined Studies Show No Association of Exposure to DDE and PCBs with Breast Cancer Risk

Background: Breast cancer in women is a serious and pervasive illness in the U.S. Overall, one in eight women will develop breast cancer at some point in her life. In some areas of the country, the risk is higher, most notably in the northeastern U.S. Some epidemiologic studies have shown associations between risk of breast cancer and exposure to DDE (the major metabolite of the banned insecticide DDT) and/or a class of chemicals known as polychlorinated biphenyls, or PCBs (once used in industry). Most of the positive studies have shown only modest increased risks while many other studies reported no association at all. To investigate this issue more vigorously, in 1993 the NIH funded five large studies. All five of these studies have reported finding no association overall between DDE, PCBs and breast cancer; however, suggestions of effects were seen in some subgroups of the studies. To investigate this matter further and to increase the capacity of the studies to detect any subtle effects, the data from the studies were combined and reanalyzed with controlled methodologies.

Advance: The combined study consisted of 1,400 case patients with breast cancer and 1,642 controls. The results do not support an association of breast cancer risk with serum/plasma levels of PCBs or DDE. The risk of breast cancer was equivalent in women who had the highest PCB and DDE levels to women with the lowest levels.

Implications: The lack of an association between PCBs and DDE and breast cancer risk does not rule out the possibility that specific PCB congeners, pesticides, or other environmental contaminants may be associated with breast cancer. However, body burdens of PCBs and DDE are not likely to explain the high rates of breast cancer in the northeastern U.S. Further research on other possible environmental risk factors is needed and is currently underway.

Laden F, Collman G, Iwamoto K, Alberg AJ, Berkowitz GS, Freudenheim JL, Hankinson SE, Helzlsouer KJ, Holford TR, Huang HY, Moysich KB, Tessari JD, Wolff MS, Zheng T, and Hunter DJ: 1,1-Dichloro-2,2-bis (p-chlorophenyl) ethylene and polychlorinated biphenyls and breast cancer: combined analysis of five U.S. studies. Journal of the National Cancer Institute 93: 768-776, 2001.

Insight into Molecular Pathways of Asthma

Background: Asthma is a serious lung disorder characterized by chest tightness, wheezing, cough, and difficulty in breathing. These symptoms are the result of inflammation and constriction in the bronchial tubes of the lung. One environmental agent that elicits these asthma symptoms is endotoxin, which is formed by common household bacteria.

The body has a number of hormone-like substances, the prostaglandins, that control constriction and dilation of the airways. Mouse models have now been developed that lack the gene coding for either Prostaglandin H Synthase 1 (PGHS-1) or PGHS-2, enabling scientists to better understand the role of these enzymes in asthma symptoms.

Advance: These researchers found that compared to normal mice, mice deficient in either PGHS-1 or PGHS-2 exhibit increased bronchoconstriction following inhaled endotoxin. These changes occur in the absence of an enhanced airway inflammatory response. These data indicate that both PGHS-1 and PGHS-2 are important in regulating the functional respiratory responses to endotoxin, but not the inflammatory responses.

Implications: Inflammation and constriction of the lung's bronchial tubes are key components of asthma attacks. Understanding the molecular pathways underlying this process, as well as how environmental agents elicit these responses, will suggest new therapeutic prevention and intervention schemes for this disease.

Zeldin DC, Wohlford-Lenane C, Chulada P, Bradbury JA, Scarborough PE, Roggli V, Langenbach R, and Schwartz DA: Airway inflammation and responsiveness in prostaglandin H synthase-deficient mice exposed to bacterial lipopolysaccharide. American Journal of Respiratory Cell and Molecular Biology 25:457-465, 2001.

Arsenic Toxicity

Background: Arsenic is a naturally occurring substance that is present throughout the earth's crust. Although history and fiction are peppered with references to the acute toxicity and lethality of arsenic, the effects resulting from chronic exposure are not as well understood. Long-term exposure to arsenic can occur through exposure to soil, water, air, and food. Exposure can also occur through the use of home remedies and herbal drugs that contain arsenic as a medication or as a contaminant. Chronic exposure to levels of arsenic that do not produce acute toxicity may cause adverse health effects including liver injury, peripheral neuropathy, or cancer. Exposures that have resulted in cancer from consumption of contaminated drinking water have been reported in several countries including Bangladesh, China, and India. Although arsenic is a well-established human carcinogen, the mechanisms by which it induces cancer remain poorly understood. Most substances that cause cancer in humans also cause cancer in laboratory animals. Arsenic is unusual because it is the only substance known to cause cancer in humans that does not produce cancer in laboratory animals. The mechanism by which arsenic causes cancer, therefore, is poorly understood. Previous work using *in vitro* methods such as cell culture has shown that arsenic can cause cellular damage leading to genotoxic effects (i.e., damage to genetic material).

Advance: Researchers showed that very short-term, transient reactive metabolites such as superoxide anions, hydroxyl radicals, and hydrogen peroxide are formed by mammalian cells treated with arsenic. If these reactive substances are not rapidly metabolized, they can cause cellular damage that may ultimately lead to genetic mutations. Additionally, removing glutathione, a substance used by cells to remove reactive metabolites, increases the mutagenic potential of arsenic 5-fold.

Implications: These data provide further evidence that reactive metabolites, particularly hydroxyl radicals produced in response to arsenic exposure, play an important role in the carcinogenic mechanism of arsenic compounds. Also, scavengers such as glutathione play a protective role by reducing hydroxyl radical concentrations. Understanding the mechanism by which arsenic causes cancer will allow us to develop methods to treat cancers caused by arsenic and to develop better methods of preventing arsenic induced cancers.

Liu SX, Athar M, Lippai I, Waldren C, and Hei TK: Induction of oxyradicals by arsenic: implication for mechanism of genotoxicity. Proceedings of the National Academy of Sciences of the USA 98(4): 1643-1648, 2001.

Maintaining Enzyme Balance for Cellular Defense

Background: Biliverdin and bilirubin are products of heme (oxygen-carrying component of blood) metabolism with approximately 70 percent to 80 percent of these products coming from hemoglobin of aging or senescent red blood cells. Red blood cells have a normal lifespan of approximately 120 days. Heme metabolism is an important metabolic process since hemoglobin must be degraded as old red blood cells are disassembled. When hemoglobin is broken down, the heme is not salvaged, but is degraded. The enzyme biliverdin reductase (BVR) catalyzes the last step in heme degradation by converting biliverdin to bilirubin. While biliverdin is a potent liver tumor promoter, it has also been shown to inhibit herpes virus and HIV-1 replication. Low levels of bilirubin, an antioxidant, have been associated with coronary artery disease. The rate of conversion of biliverdin to bilirubin is controlled by BVR and is, therefore, a very important process in cellular defense mechanisms.

Advance: Researchers have determined that BVR must be combined with phosphorus, a process known as phosphorylation, to carry out the conversion of biliverdin to bilirubin. They have shown that BVR is also a kinase (an enzyme that catalyzes the transfer of phosphate groups) and is capable of phosphorylating itself (autophosphorylation) in a physiologically reversible manner. These findings suggest that the reversible phosphorylation of BVR is an important mechanism by which activities of heme degradation products are regulated.

Implications: Enzyme phosphorylation is an important component of the regulation of cellular metabolism. Many enzymes must be phosphorylated to catalyze reactions; however, only a small fraction of them are reversibly phosphorylated. The significance of BVR phosphorylation may be related to the important role of the heme oxygenase system in cellular defense mechanisms and the complex interactions between the enzymes and products of the heme degradation pathway.

Salim M, Brown-Kipput BA, and Maines MD: Human biliverdin reductase is autophosphorylated, and phosphorylation is required for bilirubin formation. Journal of Biological Chemistry 276(14): 10929-10934, 2001.

Molecular Insights into Inflammatory Disorders such as Rheumatoid Arthritis and Crohns

Background: Inflammation exists to protects the body from infection, but it can also have a down side in the form of chronic inflammatory diseases such as rhematoid arthritis. Understanding the destructive side of inflammation is critical to controlling and treating these problems. One important cellular component is a hormone, or so-called cytokine, produced by white blood cells known as tumor necrosis factor alpha (TNF) that is involved in many of the destructive effects of certain diseases. For example, TNF is largely responsible for the cardiovascular collapse of septic shock that occurs in states of overwhelming infection. However, it also plays a role in chronic inflammatory disease. For example, in the deforming joint disease rheumatoid arthritis, it is now clear that too much TNF circulating in the blood can cause many of the symptoms and physical signs of this disease. The same is true of the inflammatory bowel disease known as Crohn's disease, and probably many other inflammatory diseases. It is important to understand how TNF exerts these harmful effects on tissues, so that novel therapies can be devised to thwart this harmful cytokine.

We now know that a cellular protein known as TTP (for tristetraprolin) is involved in the normal regulation of TNF secretion by white cells. When the gene encoding TTP was disrupted in mice, the animals developed many of the hallmarks of excess TNF in their blood, including a destructive arthritis that resembles human rheumatoid arthritis, as well as many other signs of chronic inflammatory disease. This animal model has enabled scientists to dissect the roles of the two cellular receptors for TNF in mediating the various aspects of the syndrome.

Advance: Scientists have now shown that mice deficient in both TTP and in the so-call type I TNF receptors are essentially normal, that is, they do not develop arthritis or the other characteristic inflammatory signs of TTP deficiency. Conversely, mice deficient in TTP and the other type of TNF receptor, type II, exhibit a more severe inflammatory picture than the mice deficient in TTP alone. These experiments show that the type II receptor is involved in normal physiology in a protective role, that is, its co-activation by TNF with the type I receptor normally helps to dampen some of the pro-inflammatory, harmful effects of TNF seen in the setting of TTP deficiency that are mediated through the type I receptor.

Implications: From the point of view of basic science, these experiments increase our understanding of how this important pathway of response to environmental agents is regulated by a combination of innate and external influences. However, they also have significant implications for the design of new therapies for diseases like rheumatoid arthritis. For these and other conditions of "TNF excess", inhibitors of the type I TNF receptor would be potentially very useful drugs. However, the new study suggests that *stimulators* of the type II receptor, rather than receptor blockers, might also be useful therapeutically, since such compounds would interfere in some way with the harmful effects of TNF mediated through its type I receptor.

Carballo E, and Blackshear PJ: Roles of tumor necrosis factor receptor subtypes in the pathogenesis of the tristetraprolin-deficiency syndrome. Blood 98(8): 2389-2395, 2001.

Dietary Deficiencies May Lead to Birth Defects or Childhood Cancer

Background: Although it is well known that the dietary nutrient, folate, is important for normal reproduction in women, little research on the role of folate in male reproduction has been reported. Evidence suggests that dietary deficiencies in males may cause deoxyribonucleic acid (DNA) damage in sperm and may increase the risk of birth defects and childhood cancer. Deficiencies in folate, vitamin B12, or vitamin B6 may cause uracil to be incorporated into DNA. These three vitamins are needed for converting uracil (a constituent of ribonucleic acid [RNA]) into thymine (a constituent of DNA). The incorporation of uracil into DNA may result in chromosome breaks. These breaks occur when cellular repair proteins attempt to replace uracil with thymine. Previous studies have shown that men with low vitamin C intake had more damage to their sperm DNA and that smoking caused similar effects. Smoking is known to deplete vitamin C levels.

Previous studies have shown that men whose diets are deficient in vitamin C also have an increased incidence of sperm DNA abnormalities.

Advance: Scientists at University of California at Berkeley have examined the effect of low folate on human sperm parameters in collaboration with the US Department of Agriculture. Folate deficiency in humans is correlated with decreases in sperm count and the quality of sperm. Their studies have shown that seminal plasma total folate and 5-methyltetrahydrofolate concentrations are indicators of the level of folate intake. Sperm count and quality were correlated with the non-methyltetrahydrofolate fraction of seminal plasma. Their studies have also shown that smoking depleted vitamin C levels.

Implications: Ten percent of the U.S. population has a diet deficient enough in folate to cause high DNA uracil content and the resulting chromosome breakage. An appreciable percentage of the U.S. population also has inadequate dietary intake of vitamins B12, B6, and C. People at or near the poverty level are much more likely to have dietary deficiencies which increase the likelihood that their children will suffer adverse health effects.

Wallock LM, Tamura T, Mayr CA, Johnston KE, Ames BN, and Jacob RA: Low seminal plasma folate concentrations are associated with low sperm density and count in male smokers and nonsmokers. Fertility and Sterility 75(2): 252-259, 2001.

Lykkesfeldt J, Christen S, Wallock LM, Chang HH, Jacob RA, and Ames BN: Ascorbate is depleted by smoking and repleted by moderate supplementation: a study in male smokers and nonsmokers with matched dietary antioxidant intakes. American Journal of Clinical Nutrition 71(2): 530-536, 2000.

A Protein Associated With Memory Loss in Alzheimer's Patients

Background: Alzheimer's disease is a human neurological disorder characterized by an increasing loss of cognitive function. Alzheimer's disease usually begins after age 65; however, its onset may occur as early as age 40, appearing first as memory decline and, over several years, destroying cognition, personality, and ability to function. The National Institute on Aging estimates that up to 50 percent of Americans aged 85 years or older may have Alzheimer's disease. As the baby boom generation ages, Alzheimer's disease is likely to become an even greater public health issue over the next 20 years. Although the causes of Alzheimer's disease are not yet known, researchers are making progress in determining the causes of this disease. The mental deterioration that is so devastating to patients with Alzheimer's disease is thought to be caused by deposits in the brain that are made up of a protein called beta amyloid. The deposits are formed when enzymes cut a larger protein, known as amyloid beta precursor protein, into smaller fragments. One fragment, beta amyloid, leaves the cell and can build up in plaques that can be found in the brains of patients with Alzheimer's disease.

Advance: These scientists demonstrated in rat brain that the beta amyloid binds to a receptor in the brain, thus blocking the signals, or currents, that are thought to be involved in learning and memory. Beta-amyloid blocks the function of a key receptor in the hippocampus, the seat of memory, motivation and emotion in the brain, before beta-amyloid plaques are formed.

Implications: This work provides a mechanistic explanation for the early cognitive deficits seen in patients with Alzheimer's disease long before beta amyloid plaques are formed.

Pettit DL, Shao Z, and Yakel JL□ □-amyloid1-42 peptide directly modulates nicotinic receptors in the rat hippocampal slice. Journal of Neuroscience 21 RC120: 1-5, 2001.

Male Infertility

Background: Specific maternal host factors and environmental risk factors contributing to birth defects have been studied; however, little is known about the roles of specific paternal host factors and environmental risk factors associated with birth defects. Evidence for male-mediated developmental toxicity derives from animal data and from epidemiological studies that exposures of fathers to environmental toxicants are associated with adverse consequences to the fetus and offspring. Protamines are the major DNA-binding proteins in the nucleus of sperm in most vertebrates. Many mammals have one protamine, but a few species, including humans and mice, have two. Abnormal sperm protamine levels are a common defect in infertility patients, but not in sperm of donors of known fertility. Spermatozoa from infertile men are known to exhibit increased protamine-1 to protamine-2 protein ratios. It appears that abnormal protamine levels may reflect defects of late spermiogenesis, including the ability of sperm to penetrate an egg and cause fertilization. Chromosomally defective sperm are detrimental to the developing embryo and live-born child resulting in spontaneous abortions and birth defects.

Advance: The genes for mouse protamines 1 and 2 were mutated in mice to study the function of these proteins. It was found that a full amount of both proteins is essential for sperm function. A mutation in only one of the two copies of either gene, causing reduction by one half in the amount of protein, led to defects in DNA compaction and male infertility. Protamine 2 deficiency correlates with infertility in humans, suggesting that single-copy mutations in these or other essential sperm proteins may be a cause of infertility in men with apparently normal sperm production.

Implications: Effective infertility interventions must address both male and female causes.

Cho C, Willis WD, Goulding EH, Jung-Ha H, Choi YC, Hecht NB, and Eddy EM: Haploinsufficiency of protamine-1 or -2 causes infertility in mice. Nature Genetics 28: 82-86, 2001.

Insight into Preventing Damage Caused by Balloon Angioplasty and Other Procedures

Background: The blood vessels and the heart are important oxygen-carrying components of the body. When they are denied critical levels of oxygen, which can take place with blockages due to blood clots or atherosclerotic plaques, cellular damage occurs. When this blockage is removed, (e.g., balloon angioplasty), even more local damage results, at least temporarily. Paradoxically, the cellular damage going from an oxygen-poor to an oxygen-rich environment is more detrimental than that caused by an oxygen-poor environment alone. Given that it is often medically necessary to correct oxygen-poor conditions in the body, scientists are very interested in understanding the underlying mechanisms of this hypoxia-reoxygenation injury so that it can be better controlled.

Advance: A particular metabolizing enzyme, CYP2J2, is abundant in the heart and blood vessels and is responsible for converting the body's supply of arachidonic acid to a series of compounds called epoxyeicosatrienoic acids (EETs). The EETs play important roles in protecting the heart and blood vessels from damage. A group of scientists showed that exposure of blood vessel cells from the cow to hypoxia-reoxygenation results in significant cell injury and reduced CYP2J2 expression. Importantly, they were able to prevent cell injury through maintaining levels of CYP2J2, adding more EETs, or preventing the cellular breakdown of EETs. The protective effects of CYP2J2 and its products appear to be mediated, at least in part, by antioxidant effects.

Implications: These findings give important clues to possible therapeutic interventions to prevent hypoxia - reoxygenation injury in people. This type of injury occurs in patients undergoing balloon angioplasty and surgeries, in plastic surgery using a patient's skin tissue to create flaps for reconstruction purposes, or when extremities are reattached in accident victims. Recovery in all of these situations would be enhanced if there were a means of reducing the injury that tissues incur when moving from a poorly oxygenated state to a richly oxygenated state.

Yang B, Graham L, Falck JR, Liao JK, and Zeldin DC: Overexpression of cytochrome P450 CYP2J2 protects against hypoxia-reoxygenation injury in cultured bovine aortic endothelial cells. Molecular Pharmacology 60: 310-320, 2001.

New Insights into the Development of Arthritis

Background: Both rheumatoid arthritis and osteoarthritis are characterized by inflammation of the joints and destruction of the cartilage in those joints. A particular enzyme (Cyclooxygenase-2 or Cox-2) is involved in the production of inflammatory molecules. The expression of this enzyme is increased in the synovial tissue in the joints of rheumatoid arthritis patients and in the affected cartilage in osteoarthritis patients. Consequently, Cox-2 has become a major target for the treatment of inflammatory diseases such as rheumatoid and osteoarthritis. However, the full physiological role of Cox-2 in the development of these diseases remains unclear.

Advance: A model for rheumatoid arthritis has been developed by injecting normal mice with collagen, a major component of cartilage. These mice develop an arthritis characterized by the development of antibodies to collagen. Antibody complexes become deposited in the joints resulting in inflammation and swelling of the joints followed by increased numbers of lymphocytes in the joint tissue and cartilage destruction. In addition, normal mice will develop arthritis when injected with antibodies against collagen from arthritic mice.

To understand the role of Cox-2 in the pathogenesis of arthritis, researchers injected mice that lack Cox-2 with collagen. These animals did not produce antibodies against collagen and showed no inflammation or cartilage destruction in their joints. In addition, arthritis could not be induced in the Cox-2 deficient mice by the injection of antibodies from normal arthritic mice.

Implications: Osteoarthritis and rheumatoid arthritis are common afflictions of the adult population. Therapeutic agents alleviate discomfort and maintain function for those affected. However, the pathogenesis of these diseases remain unclear. These studies shed new light on the role of Cox-2 in arthritis by showing that Cox-2 is essential for the production of antibodies as well as inflammatory molecules involved in collagen-induced arthritis. Furthermore, the presence of antibodies against collagen alone does not induce arthritis. Rather, the inflammatory products of Cox-2 are also essential for the induction of arthritis.

Myers LK, Kang AH, Postlethwaite AE, Rosloniec EF, Morham SG, Shlopov BV, Goorha S, and Ballou LR: The genetic ablation of cyclooxygenase-2 prevents the development of autoimmune arthritis. Arthritis and Rheumatism 43(12): 2687-2693, 2000.

Pathogenic Mechanism in a Novel Limb-Girdle Muscular Dystrophy

Background: All limb-girdle muscular dystrophies (LGMD) show a similar distribution of muscle weakness, affecting both upper arms and legs. Most LGMDs are caused by genetic mutations that disrupt a critical complex (the sarcoglycan complex) on muscle membranes. This sarcoglycan complex helps protect the muscle membrane (sarcolemma) from disruption during forceful contractions, though it may have other roles as well. Studies in the past decade show that sarcoglycan complexes span the sarcolemma, linking components of the extracellular matrix to the interior of the muscle cell. The muscle side of the complex binds with the protein dystrophin, which is associated with Duchenne Muscular Dystrophy (DMD). Recently, investigators identified a novel form of limb-girdle muscular dystrophy (LGMD-1C) in humans due to mutations within the genetic coding of a different membrane protein, caveolin-3. This protein is associated with caveolae, or indented regions, that represent an appendage or sub compartment of the sarcolemma.

Advance: Researchers created a mouse model that does not produce caveolin-3. These mice lack the sarcolemmal caveolae where caveolin-3 protein is normally located. In addition, analysis of skeletal muscle tissue from these mice reveals deleterious muscle changes, including variability in the size of the muscle fibers and the presence of necrotic fibers. Importantly, the researchers find that there are changes in the precise distribution of dystrophin and sarcoglycan on the muscle membrane, though concentrations are normal. This indicates that one function of caveolin is to recruit and position the dystrophin-sarcoglycan complex. Additionally, part of the muscle structure associated with excitation is disrupted. The signals that normally excite contraction are relayed from nerves and carried into muscle fibers through an elaborate maze of transverse
(T)-tubules. Mice lacking caveolin-3 have abnormalities in the organization of this system, with tubules that are considerably dilated and that are oriented longitudinally rather than transversely.

Implications: These results advance understanding the pathogenesis of LGMD-1C at a molecular level. They suggest that muscular dystrophy can develop even though concentrations of the dystrophin-sarcoglycan complex within the muscle are normal. In addition, the T-tubule system, which is involved in normal muscle excitation, is disrupted. Together the results indicate that alterations in the positioning of important elements of muscle function can result in myopathy (disease of the muscle).

Galbiati F, Engelman JA, Volonte D, Zhang XL, Minetti C, Li M, Hou H, Kneitz B, Edelmann W and Lisanti MP: Caveolin-3 null mice show a loss of caveolae, changes in the microdomain distribution of the dystrophin-glycoprotein complex, and T-tubule abnormalities. Journal of Biological Chemistry 276: 21425-21433, 2001.

Role of Angiogenesis Hormone in Skeletal Muscle Hypertrophy

Background: Skeletal muscle adapts to its pattern of usage in humans and animals. There is a significant increase in muscle size and mass (hypertrophy) in a muscle that becomes overloaded when a normally cooperating muscle is injured or torn. One study showed that functional overload resulted in a 40 percent increase in mass and a 15 percent increase in muscle protein content within five days. It is known that input from both mechanical and hormonal stimuli is necessary for optimal skeletal muscle cell hypertrophy, though the mechanisms involved are still unclear. In response to being stretched, skeletal muscle cells in culture exhibit growth only in the presence of both cell-released and serum-dependent factors. More than twenty different hormones have been linked to effects in overload-induced skeletal muscle hypertrophy. However, the role of angiogenic (capillary inducing) hormones on skeletal muscle growth has not been studied, even though one, angiotensin II (ANG II) is strongly involved in overload-induced hypertrophy of cardiac muscle cells and smooth muscle growth.

Advance: Researchers have shown a role for angiogenic factors in the response of skeletal muscle to overload. Standard overload of the animal model resulted in significantly higher total protein content in leg muscles. This hypertrophy was significantly attenuated when ANG II production was lowered by treatment with a substance known as angiotensin-converting enzyme (ACE) inhibitor. ACE inhibitor had no effect on the size of muscles which were not overloaded, indicating that the inhibitor did not act by generally inhibiting normal processes that maintain muscle size or maturation-related muscle growth. The effect of the inhibitor was much greater in a slow-twitch muscle than in a fast-twitch muscle. The effect of the inhibition could be countered by local addition of ANG II to overloaded muscle in ACE inhibitor-treated animals. This demonstrates that ANG II is necessary for optimal overload-induced skeletal muscle hypertrophy.

Implications: Angiogenesis is an important component in the body's response to increased physical activity. This study shows that a promoter of blood vessel formation, ANG II is also necessary for increased skeletal muscle mass. The mechanisms by which ANG II mediates skeletal muscle hypertrophy under conditions of overload are currently unknown. ANG II may act directly on the skeletal muscle cells themselves, indirectly through the stimulation of neighboring fibroblasts, or via the promotion of capillary angiogenesis. The mechanism of the effect remains to be clarified, particularly the difference in hypertrophic response in different muscle types.

Gordon SE, Davis BS, Carlson CJ, and Booth, FW: ANG II is required for optimal overload-induced skeletal muscle hypertrophy. American Journal of Physiology, Endocrinology, and Metabolism 280(1): E150-E159, 2001.

Molecular Causes of Painful Joints in Juvenile Rheumatoid Arthritis

Background: One of the hallmarks of rheumatoid arthritis in both juveniles and adults is the tumor-like expansion of inflamed synovial tissue, called pannus, which causes much of the joint damage in this disease. The synovial tissue is normally a thin coating on joints, but in rheumatoid arthritis the synovial cells undergo significant proliferation and inflammatory cells are brought in from the general circulation. This expansion is also supported by extensive formation of new blood vessels, which provide not only a source of nutrients for the growing pannus, but also increased access for inflammatory cells to infiltrate the synovial tissue. The molecular entities supporting this process have not been determined.

Advance: Researchers at an NIH-supported center have used a mouse model that does not have a functioning immune system to detect the molecular entities in inflamed synovial tissue. Small pieces of human tissue can be grafted into these mice without rejection of the tissue. This allows the human tissue to continue functioning but in the absence of an immune response. The researchers found that synovial tissue from patients with juvenile rheumatoid arthritis readily developed new blood vessels from cells of human origin when grafted into the mice. Several key protein receptors were identified in high numbers that are associated with promotion of new blood vessels. There was no such response when synovial tissue from patients with osteoarthritis were grafted into mice. However, a response was seen when synovial tissue from an adult with rheumatoid arthritis was studied.

Implications: This model of rheumatoid arthritis utilizing human synovial tissue grafted into a mouse will allow the study of various agents to block the growth of new blood cells and to determine their effect on inflamed synovial tissue. This offers a new route to treating rheumatoid arthritis.

Scola MP, Imagawa T, Boivin GP, Giannini EH, Glass DN, Hirsch R and Grom AA: Expression of angiogenic factors in juvenile rheumatoid arthritis. Arthritis and Rheumatism 44(4): 794-801, 2001.

New Insights into the Complex Effects of Estrogen on Bone

Background: Bone breakdown, or resorption, is a normal part of bone remodeling, in which old or damaged bone is replaced with new bone. Net loss of bone, leading to osteoporosis, occurs when bone resorption exceeds new bone formation. The most common cause of bone loss is the decline in the female sex hormone estrogen in women after menopause. Estrogen also seems to be important for the maintenance of bone mass in men, although men have much less estrogen than the male sex hormone androgen. While it remains unclear just how sex hormones influence bone remodeling, it is known that many types of cells have proteins on their surfaces called receptors, which enable the cells to respond to estrogen and androgen. But it is not clear just which cells are responsible for the effects of estrogen on bone, or even whether estrogen receptors are necessary for estrogen's effects.

Advance: Two recent reports have demonstrated that there is still much to be learned about the action of estrogen and the function of estrogen receptors. In the most surprising development, investigators have extended earlier work showing that estrogen decreases rates of controlled cell death (called apoptosis) among bone-forming cells (osteoblasts), thus increasing bone formation and preventing net bone loss. Now they find that *either* estrogen or androgen can have this anti-apoptotic effect, and that it can be mediated by *either* estrogen receptors or androgen receptors, regardless of which sex hormone is present. It appears that the effects of sex hormones on bone reflect a previously unrecognized function of the estrogen and androgen receptors, which is distinct from their familiar action on reproductive tissues. In a second report, investigators have shown that immune cells called T cells can contribute to the bone loss that occurs when estrogen levels are low. Whereas estrogen acts directly to prevent the death of osteoblasts, as described above, the moderating effect of estrogen on rates of bone breakdown is indirect. Estrogen levels apparently influence the activity of cells other than the cells (osteoclasts) that actually resorb bone. T cells produce a protein that stimulates the formation of osteoclasts, and T cells are more numerous when estrogen levels are low.

Implications: Estrogen replacement therapy, while effective, is not appropriate for all women, and cannot be used in men because of its effects on reproductive organs. Thus, there is much interest in developing drugs that would have estrogen's beneficial effects on the skeleton but would not affect other tissues. This has proven difficult because of the complexity of estrogen's action. The discovery of the novel mechanism by which estrogen prevents osteoblast apoptosis reveals a new target for drug development efforts. Although additional research will be necessary to determine whether this mechanism is also involved in estrogen's effects on other cells, such as T cells, this work opens up new possibilities for controlling bone loss.

Kousteni S, Bellido T, Plotkin LI, O'Brien CA, Bodenner DL, Han L, Han K, DiGregorio GB, Katzenellenbogan JA, Katzenellenbogan BS, Roberson PK, Weinstein RS, Jilka RL, and Manolagas SC: Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity. Cell 104: 719-730, 2001.

Cenci S, Weitzmann MN, Roggia C, Namba N, Novack D, Woodring J, and Pacifici R: Estrogen deficiency induces bone loss by enhancing T-cell production of TNF-alpha. Journal of Clinical Investigation 106: 1229-1237, 2000.

Association of COMT Genotype and Risk for Schizophrenia

Background: Both the prefrontal cortex and dopamine systems have been implicated in the pathophysiology of schizophrenia, through several lines of research in both animals and humans. Schizophrenia is heritable, but not in a manner that indicates it is caused by a single gene. Rather, it is likely that changes in several genes interact with environmental factors to increase the risk for schizophrenia.

Advance: Researchers have found a common alteration in the gene for catechol-O-methyltransferase (COMT) that controls the amount of dopamine in the prefrontal cortex. Inheritance of one form of this gene correlates with a person's difficulty in performing tasks that use the prefrontal cortex. This gene form is found much more frequently in patients with schizophrenia than in the normal population.

Implications: Although this is not a "gene for schizophrenia," it appears to alter dopamine function in the brain in a way that increases a person's risk for schizophrenia. After validating this finding, researchers will look at drugs that interact with this gene's function as a possible treatment for schizophrenia.

Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CH, Straub RE, Goldman D, and Weinberger DR: Effect of COMT $Val^{108/158}$ Met genotype on frontal lobe function and risk for schizophrenia. Proceedings of the National Academy of Sciences USA 98: 6917-6922, 2001.

Neuronal Synchronization

Background: Scientists are working to understand the processes in the brain that allow us to pay attention to some things we see while ignoring less relevant aspects of a scene. Research has shown what areas of the brain process which parts of a visual scene, but less is known about how those parts are reassembled by the visual system into a coherent picture. Further, studies have demonstrated that we do not form a complete picture, but rather only notice or pay attention to certain features of a given visual scene.

Advance: Recent studies have provided insight into how the brain filters out parts of a visual scene that it determines are not important. Researchers at NIH have shown that brain cells which are activated in response to the object of attention are "firing" in synchrony with their immediate neighbors. This synchronous firing effectively increases the gain of the attended visual item, helping the signal to the next level of brain processing.

Implications: Understanding attention at this level may allow development of better methods to improve attention in disorders such as ADHD.

Fries P, Reynolds JH, Rorie AE, and Desimone R: Modulation of oscillatory neuronal synchronization by selective visual attention. Science 291: 1560-1563, 2001.

Specifying the Brain Structures Involved in Acute Fear versus Anxious Temperament

Background: Individuals who are temperamentally anxious can be identified early in childhood and are at risk to develop anxiety and depressive disorders. Anxious temperament is characterized by a stable pattern (i.e., a trait) of shyness and inhibited behavior, and it has been shown to be associated with stable individual differences in asymmetric right prefrontal brain activity as assessed by EEG. Some previous research has suggested that the amygdala also plays a role in anxious temperament, similar to its role in acute negative affective states such as sadness and fear. However, this research has been limited; in particular, non-human primate studies involving amygdala lesions have been difficult to interpret because the lesions have been nonspecific, damaging overlying cortical and hippocampal regions as well. Recently, investigators have utilized selective fiber-sparing ibotenic acid lesions of the amygdala in rhesus monkeys in order to identify the particular emotional responses (acute fear versus anxious temperament) that are specifically associated with amygdala function.

Advance: Behavioral and physiological tests to assess acute fear and anxious temperament were administered to seventeen rhesus monkeys that underwent lesion procedures and to ten controls. Acute fear was assessed by a test for snake fear and by a social threat paradigm involving exposure to a threatening novel adult monkey. Behavior associated with anxious temperament was assessed by a human intruder paradigm that elicits individual differences in freezing and defensive hostility; these individual differences have been shown to be present early in life and to be stable over time. EEG also was used to assess right-versus left-asymmetry in prefrontal cortical activation, a physiological pattern associated with individual differences in anxious temperament. Results showed significant effects attributable to amygdala function on both tests of acute fear; compared with controls, animals with amygdala lesions demonstrated less snake fear and less submissive behavior in response to the presence of a threatening adult monkey. However, amygdala lesions did not affect measures of anxious temperament; there were no significant differences between lesion and control monkeys on defensive responses to the human intruder paradigm or on asymmetric frontal EEG patterns, and there were significant correlations from before to after surgery for both of these measures in lesioned monkeys.

Implications: These data demonstrate that the amygdala has a role in mediating acute unconditioned fear responses but not in maintaining stable individual differences associated with anxious temperament. This research represents a significant step forward in specifying both the distinctive role of the amygdala (as opposed to other brain regions) in emotion and the particular emotional responses with which is it associated. Further specification of the brain regions and pathways that mediate anxious temperament will have significant implication for understanding problematic behavior in early childhood as well as the emergence of anxiety and depressive disorders.

Kalin NH, Shelton SE, Davidson RJ, and Kelley AE: The primate amygdala mediates acute fear but not the behavioral and physiological components of anxious temperament. Journal of Neuroscience 21(6): 2067-2074, 2001.

Brain Changes in Childhood Schizophrenia

Background: Scientists have been searching for years for brain changes that correlate with psychotic symptoms that might give insight into the causes of disorders such as schizophrenia. Several studies have shown changes in the volume of various structures in the brain, correlating with the diagnosis of schizophrenia. Previously, NIH investigators had shown that in the rare form of childhood-onset schizophrenia, total brain volume is decreased, along with several other more local changes in structures of the brain.

Advance: The same intramural scientists recently examined a group of children who have psychotic symptoms, but are not diagnosed with schizophrenia. Using MRI to measure the sizes of different brain structures, they compared this group with the childhood schizophrenic group and with a group of normal children. They found that the non-schizophrenic psychotic group had brain structural changes that closely resembled those in the schizophrenic children and were significantly different from the normal children.

Implications: This study provides an early window into the differences in the brains of children with psychotic disorders as compared to normal children. Identifying these changes and their causes will help researchers to understand the mechanisms of psychotic disorders and, in the long run, develop more effective treatments.

Kumra S, Giedd JN, Vaituzis AC, Jacobsen LK, McKenna K, Bedwell J, Hamburger S, Nelson JE, Lenane M, and Rapoport JL: Childhood onset psychotic disorders: magnetic resonance imaging of volumetric differences in brain structure. American Journal of Psychiatry 157: 1467-1474, 2000.

His 'n' Her Brains: Molecular Designs for Sexual Differentiation

Background: The human race has been mystified and intrigued by differences between the sexes from the dawn of time. Today, the overwhelming success of books designed to improve communication between men and women is an acknowledgment that we perceive basic male/female differences without quite knowing why they exist. Although these communication issues may remain a mystery for some time, recent research on the neural influence of reproductive hormones has provided significant information on sexual differentiation of the brain at a molecular level. Studies of the development of a brain region involved in sexually dimorphic behaviors, the hypothalamus, during a crucial developmental window revealed that this area contains neurons that are responsive to the hormone estrogen and also to the neurotransmitter γ-aminobutyric acid (GABA), both of which appear to play roles in sexual differentiation of brain. *In vitro* studies have shown that neonatal hypothalamic neurons treated with a GABA-A receptor agonist have increased activity in the presence of estradiol, a hormone that is present in higher levels in developing male brain. These data gave rise to the important theory that GABA neurotransmission may be excitatory in neonatal males, but inhibitory in neonatal females, thus providing a molecular switch for sexual differentiation of brain.

Advance: Based on prior observations that male rats have higher levels of certain downstream signaling molecules implicated in sexual differentiation processes, the researchers went on to explore signal transduction pathways mediated by the GABA-A receptor. Treatment of neonatal rats with a GABA-A receptor agonist resulted in an increase in signaling mediated by a DNA binding molecule called the "cyclic AMP response element binding protein (CREB)" in male but not female brains, suggesting that differential gene expression may be directed in male vs female animals in response to GABA stimulation.

Implications: Elucidation of basic sexual differences in the development and response of brain areas like the hypothalamus can lead to greater understanding of factors that may indicate a gender-related predisposition to certain neuropsychiatric disorders as well as to suggest improved, gender-specific treatment.

Perrot-Sinal TS, Davis AM, Gregersen KA, Kao JPY, and McCarthy MM: Estradiol enhances excitatory gammabutyric acid-mediated calcium signaling in neonatal hypothalamic neurons. Endocrinology 142: 2238-2243, 2001.

Auger AP, Perrot-Sinal TS, and McCarthy MM: Excitatory versus inhibitory GABA as a divergence point in steroid-mediated sexual differentiation of the brain. Proceedings of the National Academy of Sciences USA 98: 8059-8064, 2001.

Imaging Glucose Metabolism to Streamline Treatment Efficacy Research in Alzheimer's

Background: Alzheimer's disease currently affects an estimated 4 million Americans, and its prevalence is projected to quadruple over the next 50 years. While treatment development remains a major focus of research efforts, identification of victims during the earliest stages of the disease, presumably when early intervention might delay or prevent disease progression, is an equally important goal. One major step in this direction was the identification of the association between Alzheimer's disease and the apolipoprotein E (APOE) ε4 allele. This association occurs in both familial and sporadic forms of the disease, and is strongest when both alleles are ε4. However, having one or both APOE ε4 alleles does not ensure that an individual will develop Alzheimer's disease, nor does it predict when onset will occur.

Advance: Based on prior research showing that Alzheimer's disease patients have declining levels of glucose metabolism in specific brain areas, researchers used positron emission tomography (PET) to characterize the cerebral metabolic rate for glucose (CMRgl) over two years in cognitively normal elderly subjects who were either ε4 heterozygotes or ε4 noncarriers and had family histories of Alzheimer's dementia. Both subject groups performed normally on a series of cognitive tests, and there was no evidence of a decline in cognitive performance in either group after two years. However, the ε4 heterozygotes had low baseline CMRgl in posterior cingulate, parietal, temporal, and prefrontal brain regions, a pattern typically seen in patients with Alzheimer's disease. Two years later, ε4 heterozygotes had suffered significant progressive declines in CMRgl in temporal cortex, posterior cingulate cortex, prefrontal cortex, basal forebrain, parahippocampal gyrus, and thalamus. While the ε4 noncarriers also showed two-year declines in CMRgl in cingulate cortex, parietal cortex, and caudate nucleus, these declines were less precipitous.

Implications: These results demonstrate that changes in brain metabolic rate can be detected in patients at risk for Alzheimer's disease before cognitive impairment occurs. The researchers used this information to develop a paradigm for estimating how many subjects of each type would be needed to reliably test the efficacy of a new treatment, thereby potentially streamlining this process.

Reiman EM, Caselli RJ, Chen K, Alexander GE, Bandy D, and Frost J: Declining brain activity in cognitively normal apolipoprotein E ε4 heterozygotes: a foundation for using positron emission tomography to efficiently test treatments to prevent Alzheimer's disease. Proceedings of the National Academy of Sciences USA 98(6): 3334-3339, 2001.

Deciphering Brain Molecules Responsible for Learning and Expressing Fear

Background: NIH-funded investigators recently ascertained that a particular brain region – the lateral nucleus of the amygdala (LA) – is an essential component of the neural circuit underlying Pavlovian fear conditioning. The LA also appears to be a crucial site of plasticity in this circuitry, although the synaptic mechanisms by which it mediates fear conditioning remain controversial. Research employing fear-conditioning experiments enables researchers to study the structure and mechanisms of fear responses in animal models. For instance, a once-neutral noise, such as a bell or tone, when paired with an aversive stimulus such as a foot shock, will come to elicit a fear response (freezing behavior). One particular molecular receptor, the NMDA receptor, is considered critical to many types of learning, including learned fear. New pharmacological tools make it possible to determine the role of specific subunits of NMDA receptor in the conditioned fear response.

Advance: Investigators examined whether the drug ifenprodil, which blocks the NR2B subunit of the NMDA receptor, disrupts the acquisition and/or expression of fear conditioning in rats. Animals were trained in one of two testing chambers. To test *tone memory*, the rats were placed in a chamber and exposed to repeating tones followed by a foot shock. To test *contextual memory*, rats were placed in a unique Plexiglass chamber with a metal grid floor, then subject to a foot shock. When animals were given ifenprodil injections (either into the general circulation via the abdomen – intraperitoneally, or i.p. – or directly into the amygdala) before fear conditioning they exhibited a significant decrease in the normal freezing behavior elicited by both the tone and context; the greater the concentration of the drug administered, the less freezing behavior they exerted. When the drug was administered prior to testing (24 hours after fear conditioning) only the highest i.p. dose prevented the expression of fear. In order to determine whether the drug is interfering with the acquisition of memories or with the consolidation of memories, scientists administered ifenprodil directly into the amygdala prior to fear conditioning, and measured their fear response 1 hour, as opposed to 24 hours later. Evidence of resultant deficits in short-term memory similar to those seen in earlier experiments involving long-term memory indicated that the drug interferes with memory acquisition.

Implications: The study indicates that short-term and long-term memories and responses to fearful events are under the control of a specific subunit (NR2B) of a receptor (NMDA) known to be important in learning and memory. It remains to be seen if the subunit is specific for the memory acquisition of fearful events, or whether it has a more general role in learning. But, the study does suggest that the learning processes, at least as associated with fear conditioning, occur very rapidly.

Rodrigues SM, Schafe GE, and LeDoux JE: Intra-amygdala blockade of the NR2B subunit of the NMDA receptor disrupts the acquisition but not the expression of fear conditioning. The Journal of Neuroscience 21(17): 6889-6896, 2001.

Adjusting Receptor Numbers at Synapses

Background: The strengthening and weakening of signaling between neurons at specialized contacts called synapses is thought to be one of the "changes" that occur in the brain during acquisition of memory. These changes in signaling strength have come to be known as synaptic plasticity, and the mechanisms underlying synaptic plasticity have been the focus of intense research in many laboratories. In the last decade or so, it has become clear that synaptic plasticity in the hippocampal formation, a brain region known to be important in learning and memory, is in part mediated by the movement of a type of molecule, the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionate) receptor, into or out of the cell membrane at synapses. How appropriate numbers of AMPA receptors are established in association with synaptic plasticity and how these receptor numbers are subsequently maintained have been examined exhaustively.

Advance: Two types of AMPA receptors are commonly found in hippocampal neurons. These two AMPA receptor types consist of 2 distinct combinations of AMPA receptor subunits. By labeling individual receptor subunits using a fluorescence marker (that can be detected optically) or a physiological marker (that can be identified on the basis of its activation properties) and introducing these engineered receptor subunits into hippocampal neurons, NIH-supported researchers now have found evidence for two distinct, subunit-specific synaptic delivery mechanisms for AMPA receptors. The first delivery process requires the presence of one of the AMPA receptor subunits (GluR1). This delivery process is activity dependent and serves to deliver additional receptors to synapses in association with synaptic plasticity. The other delivery process is mediated by a different AMPA receptor subunit (GluR2). Unlike the first delivery process, the second delivery process does not require synaptic activity and serves to recycle synaptic receptors continuously to maintain receptor numbers at a steady level.

Implications: The present findings indicate that two distinct, subunit-specific synaptic delivery mechanisms exist for AMPA-type glutamate receptors in hippocampal pyramidal neurons. They also show that in the case of AMPA receptors, a major functional difference between the distinct receptor subunits is their differing roles in synaptic receptor trafficking dynamics. In the future, it will be important to determine if this two-pathway delivery process (one mechanism to respond to external cues and control the number of receptors, and another to simply replace surface receptors to maintain receptor number) is more widely applicable to other biological processes where establishing and maintaining the appropriate number of cell surface receptors is critical. It also will be important to identify and explore molecules that signal the levels of receptor numbers that need to be established/maintained. Finally, these studies suggest that it may be possible to develop effective drugs that target different facets of synaptic receptor trafficking dynamics.

Shi SH, Hayashi Y, Esteban JA, and Malinow R: Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. Cell 105: 331-343, 2001.

Neurogenesis: New Memories Require New Cells

Background: Evidence for adult neurogenesis has been reported since 1965. It has been a demonstrable finding in birds since 1983, but only recently has the evidence been extended with confidence to rats, non-human primates, and humans. But do the new neurons play a crucial role in brain function or behavior? Most of the recent mammalian evidence has focused on studies of the hippocampus, a brain region known to be involved in learning, memory, and adaptations to stress. Work with rats suggests that thousands of new neurons are produced each day in the hippocampus although most die. The evidence is strong that in the course of learning the production of new neurons is enhanced, yet it has not been clear if the new neurons are actually involved directly in the formation of memories in the hippocampus.

Advance: The investigators found that a form of associative learning, called trace conditioning, which depends on the hippocampus, requires newly generated neurons. Treatments that reduced the numbers of newborn neurons by approximately 80 percent were effective in impairing trace conditioning. Since these studies were done *in vivo*, it was possible to observe the recovery of normal neurogenesis in the same animals. When trace conditioning was performed again there was a clear recovery of memory function. It is clear that some of the new neurons in the hippocampus must participate in the formation of new memories.

Implications: The evidence of neurogenesis in adults has dramatically changed the way we think about brain plasticity and the potential medical applications of this knowledge. Understanding the functions of neurogenesis may lead to important contributions to medical interventions involving brain repair including repair from injury, as well as from degenerative processes that may involve numerous psychiatric and neurological disorders. Through this research it may become possible to restore normal function by introducing healthy neurons into brain circuits that have become dysfunctional. This research is also teaching us about the important role behavior and experience may play throughout the lifespan in changing the structure and function of the brain itself.

Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, and Gould E: Neurogenesis in the adult is involved in the formation of trace memories. Nature 410: 372-376, 2001.

Synaptic Vesicle Protein 2 (SV2) as a Key Regulator of Neurosecretion

Background: Understanding neuronal functioning at a molecular level is fundamental to elucidating the origins of mental disorders. Neurosecretion, the regulated exocytosis, or discharge from cells, of neurotransmitters, is a primary function of neurons that is altered with experience and in disease. Neurosecretion is mediated by a specialized cycle of vesicle formation, filling, and fusion that shares many features with other forms of membrane trafficking in eucaryotic cells. Despite the extensive similarities, several features make neurosecretion unique. These include the strict dependence of synaptic vesicle fusion on calcium; the low probability of fusion in most neurons; the speed with which synaptic vesicles fuse; and the fact that synaptic vesicle fusion is plastic, i.e., influenced by previous secretory activity. These features are produced by the addition of regulatory molecules to the basic trafficking machinery. Recent studies have sought to identify the molecular events that produce and regulate neurosecretion, in particular how Synaptic Vesicle Protein 2 (SV2) contributes to the regulation of exocytosis. SV2 proteins, of which there are three known isoforms termed A, B and C, display characteristics expected of regulators unique to neurosecretion. For example, SV2s are expressed exclusively in neurons and endocrine cells and have no amino acid sequence homolog in yeast. The sequence and predicted membrane topology of the SV2 proteins shares significant similarity with a large family of membrane transporter proteins that conduct small molecules such as sugars, TCA-cycle intermediates, and neurotransmitters. Classical exogenous expression approaches have not been successful in demonstrating SV2 to be a transporter. This, along with the fact that proteins with multiple membrane domains and multiple glycosylation sites are difficult to work with using standard biochemical/cell biological approaches, indicated that a reverse genetic approach would be the best way to identify SV2's physiological and molecular actions.

Advance: The mutant mice in which the SV2A and SV2B genes were disrupted have been crossed to produce SV2A/B double knockouts. Studies of these mice have revealed that SV2 is an essential protein and support the conclusion that it is involved in the regulation of vesicle fusion. Disruption of the gene encoding the most widely expressed SV2 isoform, SV2A, produces mice that fail to grow normally, develop debilitating seizures, and die by three weeks of age, a phenotype suggesting multiple neurological and endocrine deficits. SV2A isoform was expressed in most GABAergic inhibitory neurons, and SV2A knockout mice revealed decreased action potential-dependent inhibitory neurotransmission but normal action potential-independent neurotransmission in the hippocampus. The decreased neurotransmission was not due to changes in the number or morphology of either synapses or synaptic vesicles, indicating that SV2 is not a structural component of synapses or required for synaptic vesicle biogenesis. These results suggested that SV2 acts instead as a positive modulator of calcium-stimulated exocytosis.

Implications: This study has utilized molecular genetic approaches to demonstrate that SV2 is one of the key molecules regulating the number of secretory vesicles. Changes in synaptic efficacy mediated by SV2 may be a physiologically important mechanism underlying learning and memory.

Tao X, and Bajjalieh SM: SV2 modulates the size of the readily releasable pool of secretory vesicles. Nature Cell Biology 3: 691-698, 2001.

Identification of a Potential Vulnerability Gene for Autism

Background: Autism is a developmental disorder of the brain that typically begins in infancy and is evident by the age of three. Distinguishing features of autism include a lack of social interaction or responsiveness, limited verbal communication and ritualized interests and behaviors. Estimates of the percentage of the population subject to autism range from 1 in 2,500 to 1 in 500 individuals as having the disorder. The genetic, or heritable, component is thought to account for as much as 90 percent of the liability to autism. Evidence to date is most consistent with the involvement of multiple genes, each of small effect, that together with nongenetic factors produce vulnerability. Several regions of the genome have been linked to autistic disorder, although none have been unambiguously confirmed and no specific genes have been identified to date. However, genetic abnormalities have been identified in a small number of autistic patients. For instance, a breakpoint was found in one section of DNA in one patient (Vincent et al., 2000).

Advance: Scientists studied a specific gene (called *WNT2*), which is located adjacent to the previously identified DNA breakpoint, to determine if it had a role in autism. *WNT2* is a likely "candidate gene" for autism because it is known to play a critical role in the development and behavior of many animals. Additionally, mice that lack a signaling molecule (called *disheveled*) required by *WNT2* exhibit abnormal social behaviors, reminiscent of humans afflicted with autism. The mice display reductions in general social interactions, huddling during sleep, and other grooming and maternal behaviors. Families with autistic members were recruited to participate in the study and all were clinically assessed and classified according to speech and language characteristics. Linguistic problems have been localized to the region of the gene in question, and some autistic individuals – and, in some instances, the parents of autistic individuals – have severe language impairment. The researchers found that two families contained sequence mutations in *WNT2* that cause a functional change in its product. The mutations occurred in one of the parents, and in both affected children, but not in the unaffected children or in the 160 control subjects. The researchers also determined that autistic individuals having the most severe language deficiencies had a specific sequence variation that was significantly linked to autism. Furthermore, they confirmed the presence of *WNT2* in a human brain region (the thalamus) important for the integration of information.

Implications: Several types of genetic studies now have indicated that a specific region of DNA, near the *WNT2* gene, is at least partially involved in autism. While this "specific region" is rather large, the corroboration of different groups using different methods is encouraging and may prove to be one of the first "real" linkages to a mental disorder. Considering that other language and speech disorders are associated with this region, the WNT2 gene may be the first of many genes involved in autism. Hopefully, the identification of each gene involved in the disorder will lead synergistically to the elucidation and function of other vulnerability genes and, ultimately, to earlier diagnosis and effective treatments for individuals with autistic disorder. [secondary – diagnosis]

Wassink TH, Piven J, Vieland VJ, Huang J, Swiderski RE, Pietila J, Braun T, Beck G, Folstein, SE,. Haines JL, and Sheffield VC: Evidence supporting WNT2 as an autism susceptibility gene. American Journal of Medical Genetics 105: 406-413, 2001.

Cytoskeletal Defect Causes Alexander Disease

Background: Alexander disease is a rare brain disorder whose cause remains a mystery. The disease is one of a group of disorders called leukodystrophies, from leuko meaning white and dystrophy meaning degeneration. The degeneration of white matter in these diseases reflects the loss of nerve fibers' electrical insulating covering, which gives white matter its appearance. This leads to widespread disruptions of brain activity. Most children with Alexander disease die young after experiencing seizures, mental retardation, and other problems.

Advance: The cytoskeleton is the internal structural support system of all cells and plays an active role in many cellular functions, such as transporting material to where it is needed within cells. Basic scientists trying to understand the cytoskeleton developed a strain of genetically engineered mice that overproduce the protein GFAP (glial fibrillary acidic protein). GFAP is a critical component of the cytoskeleton in astrocytes, a type of glial, or supporting cell, in the brain. Mice that produced too much GFAP died young. Researchers examining the brains of these mice noticed that astrocytes contained abnormal clumps of proteins that looked indistinguishable from protein aggregates that are hallmarks of brain cells in children who die from Alexander disease. Subsequent study of DNA samples from patients with Alexander disease confirmed that mutations in the gene for GFAP are responsible for most cases of the disease.

Implications: The findings provide the first good clues about what goes wrong in Alexander disease. The animal model will help scientists study how the disease develops and test strategies to stop it. The new insights from this research also bring together two broader trends in neuroscience: a growing appreciation for the importance of glial cells in health and disease and increasing appreciation of how the cytoskeleton actively contributes to many types of cellular functions.

Brenner M, Johnson AB, Boespflug-Tanguy O, Rodriguez D, Goldman JE, and Messing A: Mutations in GFAP, encoding glial fibrillary acidic protein, are associated with Alexander disease. Nature Genetics 27: 117-120, 2001.

How Huntington's Disease Kills Brain Cells

Background: Huntington's disease is a fatal neurodegenerative disorder. Although the inherited gene defects are present at birth, typically movement problems, personality disruption, and mental deterioration first become apparent in middle age and progressively worsen over several years as more brain cells die. In 1993, after a decade long search, a team of scientists found the gene, that, when defective, causes this disease. The gene carries an abnormal "triplet repeat"- a three letter "word" of the genetic code is repeated many times. So, the defective protein produced by the gene, called huntingtin, carries many repeats of the protein building block glutamine, which the repeated triplet signifies. Researchers suspect that these extra glutamines damage the brain, but do not know how.

Advance: Scientists have discovered that the abnormal huntingtin disrupts brain cells by entangling a protein called CBP. CBP is a critical regulatory molecule, of the cells' controls for turning on other genes. In Huntington's disease, CBP sticks to the abnormal huntingtin protein and cannot do its job, so genes that make growth factors and other proteins critical for the survival of nerve cells do not get turned on. The research team confirmed this scenario through studies of nerve cells in culture, in mice genetically engineered to get Huntington's disease, and by examination of post-mortem brains from human patients. The research team also demonstrated that adding a modified version of CBP that does not stick to huntingtin can overcome the deleterious effect of the Huntington's gene, at least in cultured cells.

Implications: Finding drugs that block the binding of CBP to huntingtin may be a new strategy to slow the course of Huntington's disease. There are at least eight other neurodegenerative disorders caused by glutamine repeats in different genes that may damage brain cells through similar mechanisms. Abnormal aggregations of proteins are also a common theme in other neurodegenerative diseases, such as Alzheimer's and Parkinson's, so the potential implications may be broader still. Building on these results will depend on answering questions such as how the huntingtin and CBP, which are normally in different compartments of the cell, get together in the first place.

Nucifora FC Jr, Sasaki M, Peters MF, Huang H, Cooper JK, Yamada M, Takahashi H, Tsuji S, Troncoso J, Dawson VL, Dawson TM, and Ross CA: Interference by Huntingtin and Atrophin-1 with CBP-mediated transcription leading to cellular toxicity. Science 291: 2423-2428, 2001.

Genetic Findings Lead to Clues about Parkinson's Disease

Background: What causes nerve cells to die in Parkinson's disease is still not fully understood. Most cases of Parkinson's are "sporadic," that is, not inherited or caused by an obvious environmental insult. However, the clues from rare inherited forms of Parkinson's disease are stimulating progress in understanding the more common type as well. An NIH Workshop in 1995 led directly to the first discovery of a gene defect that can cause Parkinson's. The defective gene codes for the protein alpha-synuclein. Subsequent study showed that synuclein is a major part of Lewy bodies in the common form of the Parkinson's as well as people with rare synuclein mutations. Lewy bodies are abnormal clumps of proteins found in brain cells that are a hallmark of Parkinson's disease. Studies in Japan of families with a rare form of juvenile Parkinson's disease identified another gene defect, in a protein named parkin, and other evidence implicated a protein called UCH-L1 in at least one family with inherited Parkinson's. Each of these finding provides important clues about processes that might contribute to Parkinson's, but research on each of these proteins has proceeded independently.

Advance: A new study shows that the proteins parkin and alpha-synuclein normally interact in healthy nerve cells. Parkin is involved with a normal cellular process that targets cellular proteins for disposal and recycling by tagging them with a short protein called ubiquitin. Parkin normally attaches these critical ubiquitin tags to synuclein. One possible interpretation is that defective targeting of alpha-synuclein for degradation by parkin results in the accumulation of aggregates of synuclein, along with ubiquitin and other proteins, and these aggregates kill dopamine nerve cells. Although the role of UCH-L1 was not studied directly by these investigators, this protein is also part of the ubiquitin system for recycling proteins.

Implications: Abnormal aggregation of proteins has emerged as a common theme in neurodegenerative disorders. Mutant forms of proteins form aggregates that, in one way or another, have been implicated in Alzheimer's, Huntington's and spinocerebellar ataxias as well as Parkinson's disease. Developing drugs that interrupt the aggregation process may be a strategy for slowing the progression of these disorders. These results highlight another general trend in the study of neurological disease. In disorders such as ALS, Alzheimer's and Parkinson's, that are usually sporadic, the less common inherited forms are helping researchers identify proteins and cellular processes that may be involved in all forms of the disease.

Shimura H, Schlossmacher MG, Hattori N, Frosch MP, Trockenbacher A, Schneider R, Mizuno Y, Kosik KS, and Selkoe DJ: Ubiquitination of a new form of □-synuclein by parkin from human brain: implications for Parkinson's Disease. Science 293: 263-269, 2001.

Understanding How Trauma Damages Brain Cells

Background: According to the U.S. Centers for Disease Control and Prevention more than 5 million Americans suffer long term disabilities as a result of traumatic brain injury. One of the most important consequences of trauma is widespread damage to nerve fibers, termed diffuse axonal injury. Although severe incidents may have sufficient force to immediately tear nerve fibers, much of the damage follows a slower time course following the traumatic event. Scientists have suggested that the entry of calcium ions into nerve fibers may play a pivotal role in this process because tight control of calcium within cells is critical to controlling many cellular functions, but the role of calcium has not been demonstrated directly.

Advance: Scientists studied the mechanical forces imposed on nerve cells and fibers in the intact brain. Based on this information they developed a cell culture model that mechanically stretched nerve fibers in a manner that mimics what occurs during trauma in the intact brain. Using the cell culture model, researchers made measurements that would not be feasible in intact brain. After injecting a dye that measures calcium in living cells, the scientists demonstrated that stretch injury leads to entry of calcium into cells. However, further studies showed that the route of calcium entry was not what had been expected. The initial event is entry of sodium ions, which then indirectly leads to an increase in calcium. The entry of sodium ions activates a sodium-calcium exchange molecule and leads to electrical changes in nerve fibers that open calcium pores in the cell membrane.

Implications: The findings suggest that therapy to prevent the entry of sodium ions following trauma or to counteract the secondary changes in calcium may reduce the slow damage to nerve fibers that follows brain and spinal cord trauma. Previous experiments with sodium channel blockers on spinal cord injury in rats support this idea.

Wolf JA, Stys PK, Lusardi T, Meaney D, and Smith DH: Traumatic axonal injury induces calcium influx modulated by tetrodotoxin-sensitive sodium channels. Journal of Neuroscience 21: 1923-1930, 2001.

Teng YD and Wrathall JR: Local blockade of sodium channels by tetrodotoxin ameliorates tissue loss and long-term functional deficits resulting from experimental spinal cord injury. Journal of Neuroscience 17: 4359-4366, 1997.

Mathematical Model Enables Estimation of Cost-Effectiveness of HIV/AIDS Treatment

Background: In developed nations, antiretroviral therapy with a combination of three drugs is now the standard of care for treatment of human immunodeficiency virus (HIV) infection and Acquired Immunodeficiency Syndrome (AIDS) and has significantly decreased illness and death among people with advanced HIV infection and AIDS. However, health care disparities exist due to the cost of combination antiretroviral treatment. The major challenge in treatment is to prevent morbidity and mortality due to HIV/AIDS by maximizing efficacy in a cost-effective manner.

Advance: NIH-funded researchers have developed a computer-based mathematical model that can simulate the course of HIV/AIDS and the cost of treating the disease using assumptions about the stage of disease at which treatment is started, the type of treatment provided, and the patient's response to the treatment. In their primary analysis, researchers used the model to assess the cost effectiveness of combination antiretroviral therapy for HIV-infected patients with advanced disease, by running 1 million computer simulations of HIV disease. They compared the clinical and cost outcomes for very ill individuals, with and without combination therapy. The precise disease state and other characteristics assigned to each simulated patient were drawn randomly from data collected during a large trial conducted by the NIH-funded AIDS Clinical Trial Group. The researchers learned that the projected life expectancy for patients who did not receive combination therapy is only 1.53 years, when adjusted for quality of life. In contrast, the projected life expectancy for patients receiving combination therapy is 2.91 years (quality adjusted). This 1.38 years gain in life expectancy is clinically significant for people with advanced HIV/AIDS. The medical cost per patient who received combination therapy was $31,840 higher than the cost per patient who did not receive it. (Medical costs included doctors visits, tests, treatment of symptoms and opportunistic infections, hospitalization, and other disease related expenses.) Thus, the researchers estimated the incremental cost per life-year (quality adjusted) at $23,000. This finding shows that combination therapy for HIV/AIDS is more cost effective than many accepted therapies for other diseases, including radiation for early-stage breast cancer, treatment of genetically-based high cholesterol, and dialysis in patients expected to live for less than 6 months. The researchers extended the computer model to patients whose disease was less advanced and found that the incremental cost per quality-adjusted life-year of combination therapy can be as low as $13,000. The model accounts for variations in drug costs so that cost-effectiveness can be forecast under assumptions of higher and lower costs. It also can accommodate future knowledge about HIV/AIDS pathogenesis, e.g., how various test results predict the course of disease.

Implications: The mathematical model for estimating the cost-effectiveness of HIV/AIDS treatment provides evidence for the value of combination antiretroviral therapy. Information derived from the model will aid policy makers in decision-making regarding support for HIV/AIDS treatment programs.

Freedberg KA, Losina E, Weinstein MC, Paliel AD, Cohen CJ, Seage GR, Craven DE, Zhang H, Kimmel AD, and Goldie SJ: The cost effectiveness of combination antiretroviral therapy for HIV. The New England Journal of Medicine 344: 824-831, 2001.

Gene Variant is Associated with Reduced Risk for Heart Attack

Background: Atherosclerosis, a major cause of death in industrialized countries, is the progressive narrowing and hardening of arteries over time that can cause heart attacks when it affects arteries that supply blood to the heart (coronary arteries). This narrowing occurs, to some degree, with aging, but major risk factors for atherosclerotic heart disease – smoking, high blood pressure, high cholesterol, diabetes, and a family history of heart attack – accelerate the process. Researchers now believe that inflammation plays a major role in the onset and progression of the disease. The fact that atherosclerosis runs in families suggests that the genes a person inherits contribute to the risk of developing the disease. Differences in some DNA sequences among individuals (gene variants) are associated with inflammation of blood vessels and, therefore, could affect the risk of atherosclerotic heart disease. One of these gene variants, known as CX3CR1-I249, causes the CX3CR1 protein on the surface of certain white blood cells to be produced in lower amounts. The CX3CR1 protein responds to a chemical signal that attracts inflammation-causing white blood cells to specific places in the body, including atherosclerotic lesions in blood vessels, and causes these cells to stick to the vessel wall. Scientists thought that people who have the I249 variant gene for the CX3CR1 protein, independent of established coronary disease risk factors, might be somewhat protected from atherosclerosis.

Advance: A team of NIH and French researchers found that the I249 variant of the CX3CR1 gene is associated with a markedly reduced risk of heart attack, angina, and other coronary artery problems. They demonstrated this by comparing the prevalence of the variant in people who have had a heart attack to people who have not. The two groups of people were similar in terms of age and gender distribution. As expected, risk factors for heart disease (including current smoking, high blood pressure, high cholesterol, diabetes, and obesity) were more frequent among people who have had a heart attack. But as the researchers predicted, the I249 variant was less common in people who have had a heart attack, even after adjusting for differences between the two groups in the other risk factors. Moreover, the I249 variant of CX3CR1 was less common in people in whom coronary artery narrowing was evident as determined by specific x-ray studies, and in those individuals with poor coronary artery function. The association of the I249 variant with reduced risk of atherosclerotic heart disease indicates that this gene variant may be a protective factor.

Implications: The results of this study open a new direction in basic research of atherosclerotic heart disease. This research points to normal CX3CR1 as a potential regulator of inflammation in atherosclerosis. Drugs that block CX3CR1 might help to prevent the onset and slow the progression of atherosclerosis.

Moatti D, Faure S, Fumeron F, Amara MEW, Seknadji P, McDermott DH, Debré P, Aumont MC, Murphy PM, de Prost D, and Combadière C: Polymorphism in the fractalkine receptor CX3CR1 as a genetic risk factor for coronary artery disease. Blood 97: 1925-1928, 2001.

McDermott DH, Halcox JPJ, Schenke WH, Waclawiw MA, Merrell MN, Epstein N, Quyyumi AA, and Murphy PM: Association between polymorphism in the chemokine receptor CX3CR1 and coronary vascular endothelial dysfunction and atherosclerosis. Circulation Research 89: 401-407, 2001.

Scientists Elucidate Structure of Mysterious Pathogen-Reacting Protein

Background: The immune system begins its attack on invading pathogens – such as bacteria, viruses, and parasites – when immune cells recognize "foreign" molecules, called antigens. The immune cells that recognize antigens are termed B and T lymphocytes. B cells produce antibodies, antigen-recognizing proteins, that are released into the bloodstream, where they attach to certain small, free-floating or cell surface antigens and help clear pathogens from the body. T cells have molecules or receptors on their surface that facilitate antigen recognition. Two types of T-cell receptors have been described. One type of T-cell receptor is the □□ T-cell receptor (TCR). □□ TCRs recognize small pieces of antigens bound to the surface of other immune cells. The result of this interaction is the initiation of a coordinated immune response to the antigen. Another T-cell receptor involved in antigen recognition is the □□ TCR. After decades of research, scientists have determined the structure and function of antibodies and □□ TCRs, but □□ TCRs have remained largely a mystery. Up to half of the T cells in the skin, gastrointestinal tract, and reproductive system are □□ T cells. The number of T cells in the body that have □□ TCRs increases following infection with pathogens that cause tuberculosis, malaria, and food poisoning, indicating that these receptors play some role in immune responses directed against these disease-causing organisms.

Advance: NIH scientists, working with researchers in France, employed a process called x-ray crystallography to determine the three-dimensional molecular structure of a □□ TCR, thereby uncovering some clues as to how it functions. Specifically, the scientists determined the spatial orientation of the four protein subunits that comprise the receptor molecule. Interestingly, they found that although the □□ TCR structure is similar to that of the □□ TCR, portions of the □□ TCR also are structurally similar to the antigen-binding region of an antibody molecule. In addition, the investigators located a site on the □□ TCR that might bind phosphoantigens – small antigens containing a chemical group called a phosphate – that are released by the pathogens that cause tuberculosis, malaria, and several other diseases.

Implications: These findings suggest that □□ TCRs are involved in immune responses triggered by the binding of phosphoantigens from pathogens that cause tuberculosis, malaria, and other diseases. Knowing the shape and structure of the □□ TCR will help in understanding the unique roles that these receptors play in the immune response and may aid the production of vaccines designed to stimulate immune responses against these pathogens.

Allison TJ, Winter CC, Fournié JJ, Bonneville M, and Garboczi DN: Structure of a human γδ T- cell antigen receptor. Nature 411: 820-824, 2001.

New Model for Hepatitis C Virus Replication will Advance Basic Research and Drug Development

Background: Hepatitis C virus (HCV), which infects almost 4 million Americans, is the most common chronic blood-borne infection in the U.S. Worldwide, an estimated 170 million people are chronically infected with HCV, and 3 to 4 million people are newly infected each year. Patients with chronic HCV infection often do not know they carry the virus because they can remain without symptoms for years. When the disease is finally diagnosed, patients may have already developed serious liver damage or chronic liver disease. No vaccine is available to prevent HCV, and treatments for chronic infection have limited effectiveness and significant side effects. Efforts to design better drugs to treat HCV have been hampered by the lack of cell culture systems robust enough to actually study the dynamics of HCV replication in the laboratory.

Advance: Researchers have now developed a model cell culture system having high enough levels of HCV replication to enable study of the dynamics of HCV replication. They established this model system by identifying mutations in a region of the HCV genome that codes for a protein called NS5A, which significantly enhances the replication efficiency of HCV. The researchers validated the model by demonstrating that interferon, a substance currently licensed as a therapy for HCV, could significantly inhibit the viral replication seen in the system.

Implications: This model cell culture system should be extremely valuable for defining more precisely the details of HCV replication as well as for studying the interactions of the virus and the host cell over time. Since the system is robust, it should help researchers more rapidly discover, design, and evaluate new drug therapies for HCV. In addition, the identification of mutations in the NS5A protein, which affect the ability of the virus to replicate, may aid in the development of an HCV attenuated vaccine (a vaccine containing weakened virus that normally does not cause disease but triggers an immune response that protects against the disease-causing virus).

Blight KJ, Kolykhalov AA, and Rice CM: Efficient initiation of HCV RNA replication in cell culture. Science 290: 1972-1974, 2000.

Amyloid- β and Implications for the Alzheimer's Disease Process

Background: Alzheimer's disease causes progressive deterioration of brain functions including impairment of memory and judgment. Alzheimer's is a major health problem, the risk of which increases with age. In this disease, a protein known as amyloid-β (amyloid-beta) forms abnormal aggregates in the brain called senile or neuritic plaques that result in loss of mental function. Although the mechanism by which amyloid- β contributes to Alzheimer's disease is not fully understood, scientists do know that the aggregates of this protein become associated with dying nerve cells and can produce inflammation. Aspirin-like drugs, which can reduce inflammation, appear to slow down the mental deterioration caused by Alzheimer's disease. Research had indicated that amyloid- β is not just an inactive protein but, in fact, has a variety of effects on cells, including some effects that lead to inflammation. In particular, amyloid- β can attract inflammation-producing white blood cells, causing them to move into specific brain regions, and thereby trigger production of highly reactive oxygen-derived free radicals that can damage nearby cells, including nerve cells. However, the mechanism of amyloid- β action on cells was not fully defined.

Advance: Researchers have demonstrated that amyloid- β can bind to and potently activate specific receptors on some immune system cells. The receptors, FPR2 in mice and its human counterpart, FPRL1R, are proteins found on the cell surface. Researchers have now demonstrated that amyloid- β activation of immune cells induces both chemotaxis (directed movement of cells towards a chemical) and the production of oxygen-derived free radicals in cells grown in the laboratory. In addition, researchers determined that the FPR2 receptor for amyloid- β is present in mouse brain tissue and on cells derived from mouse brain tissue. Consistent with these findings, other researchers found evidence that FPRL1R is present on inflammation-causing cells infiltrating senile plaques in brain tissues from patients with Alzheimer's disease.

Implications: Future research on this receptor should provide further insights on how the presence of amyloid- β in the brain causes inflammation and disease. This new direction in basic research on Alzheimer's disease may lead to new treatments for patients. Specifically, the discovery may lead to new therapies that could slow progression of the disease.

Tiffany HL, Lavigne MC, Cui YH, Wang JM, Leto TL, Gao JL, and Murphy PM: Amyloid-_ induces chemotaxis and oxidant stress by acting at formylpeptide receptor 2, a G protein-coupled receptor expressed in phagocytes and brain. Journal of Biological Chemistry 276: 23645-23652, 2001.

"Front Line" Immune Cells Have a Surface Protein That Is Critical for Responding to a Specific Virus

Background: Natural killer (NK) cells serve as a first line of immune defense against infection from disease-causing microorganisms (pathogens) such as viruses and bacteria. NK cells destroy cells infected with these pathogens before other immune responses, such as the production of antibodies, are mobilized. Unlike the later immune responses, which require immune cells to recognize a specific "foreign" molecule, the NK cell response generally has been thought to be nonspecific. Yet NK cells appear to respond specifically against certain pathogens. One observation that supports the idea of NK cell specificity is that people with NK cell deficiency suffer from certain recurring infections, especially with herpesviruses such as cytomegalovirus. Until now, researchers did not know exactly how NK cells recognize and respond to infected cells.

Advance: Investigators have discovered a protein called LY-49H on the surface of mouse NK cells that is critical for responding to cells infected with a specific virus – murine (mouse) cytomegalovirus (MCMV). The researchers predicted that LY-49H binds to a molecule on the surface of a MCMV-infected cell to activate the NK cells to release substances that kill the virus-infected cell. To identify LY-49H as the protein critical in the interaction with MCMV, the researchers used a strain of mouse that is susceptible to MCMV. They found that these mice have a defect in the gene that directs production of the LY-49H protein, resulting in an inability to make this protein. To provide further evidence that the LY-49H protein is critical for resistance to MCMV, the scientists introduced antibodies specific to LY-49H into resistant mice and then infected the mice with MCMV. The mice were susceptible to the virus. This showed that the antibodies to LY-49H had blocked activation of the NK cells that would have targeted MCMV and, thus, provides evidence of the involvement of the LY-49H surface protein in protection against MCMV.

Implications: This research sheds new light on the early, rapid response of the immune system to invading microbes. The results indicate that a particular NK cell surface protein is involved in the response to a specific virus. Future research will examine the role of the LY-49H protein – and perhaps other, related proteins – in responding to other viral infections. Understanding the details of how NK cells respond to specific viruses should facilitate the development of improved antiviral drugs and vaccines.

Brown MG, Dokun AO, Heusel JW, Smith HRC, Beckman DL, Blattenberger EA, Dubbelde CE, Stone LR, Scalzo AA, and Yokoyama WM: Vital involvement of a natural killer cell activation receptor in resistance to viral infection. Science 292: 934-937, 2001.

Scientists Identify Gene Activated by Vaccine Enhancers

Background: Vaccines are designed to prime the immune system to respond to a subsequent exposure to a microbe such as a bacterium, virus, or parasite, and thereby provide protection against infectious diseases. One approach to vaccine development is to identify a specific microbial protein that trigger an immune response in the host, purify the protein, and inject it as a vaccine. A disadvantage of this approach is that the immune responses to these proteins are often weak and short-lived. In order to improve immunity, some vaccines are combined with adjuvants, substances that enhance the immune response. Little is known about how adjuvants work, although researchers know that adjuvants can increase the life expectancy of the subset of T cells (a type of immune cell) that orchestrates the response against a specific disease-causing microbe.

Advance: NIH scientists have discovered that adjuvants can activate a gene within T cells that is responsible for the production of a protein called Bcl-3, and that this protein increases the life span of these T cells. Greater longevity for these T cells translates into an increased immune response to the microbial protein in a vaccine. The researchers began by comparing the activity of a large array of genes in T cells that had or had not been exposed to adjuvants. They found that two different types of adjuvant could increase Bcl-3 production, suggesting that increased Bcl-3 production may be a common feature of adjuvant activity. The scientists then did a series of experiments that showed that Bcl-3 activation prevents the death of the T cells both in the test tube and in mice.

Implications: Knowledge of how adjuvants work at the genetic level may help in the design of improved and new vaccine adjuvants. Such information also furthers understanding of the molecular basis of effective immune responses, which may aid in the design of new vaccines. Future studies will examine the details of how Bcl-3 works to promote T-cell survival.

Mitchell TC, Hildeman D, Kedl RM, Teague TK, Schaefer BC, White J, Zhu Y, Kappler J, and Marrack P: Immunological adjuvants promote activated T cell survival via induction of Bcl-3. Nature Immunology 2: 397-402, 2001.

Researchers Identify Immune Cells that Help Transmit HIV Throughout the Body

Background: Human immunodeficiency virus (HIV) primarily infects a key type of immune cell known as a CD4 positive (CD4+) T cell, so-called because it bears a protein called CD4 on its surface. HIV binds to the CD4 molecule, facilitating entry into these cells. In most cases, viral entry into CD4+ cells is followed by rapid viral replication and release of new virus, killing the host cell in the process. The deterioration of immune function characteristic of HIV infection is the result of viral attack on CD4+ T cells, which are critical for initiating immune responses to infections. In other cases, HIV enters CD4+ cells and remains inside in an inactive or resting state. More recently, several groups of researchers have determined that HIV also can bind to types immune cells that lack the CD4 molecule. Although HIV binding to CD4 negative cells does not appear to lead to viral entry into these cells, the virus can circulate throughout the body in the bound state and potentially infect non-infected CD4+ cells, especially T cells.

Advance: NIH scientists have discovered that the B cell (antibody-producing immune cell that lacks the CD4 molecule) can bind to HIV and can readily transmit the virus to CD4+ cells. The researchers isolated B cells from the lymph nodes and blood of people with chronic HIV infection and found that HIV was attached to the external surface of these cells in each case. They then demonstrated that the HIV attached to B cells could infect T cells grown in the laboratory, indicating that the virus bound to B cells remains infectious. Further, they determined that HIV binding to the B-cell surface involves a B-cell protein called CD21, which is involved in activating B cells to produce antibodies. Interestingly, CD21 levels on the B cells of HIV-positive patients were significantly lower than CD21 levels on the B cells of HIV-negative individuals.

Implications: Because B cells circulate in the bloodstream and migrate through tissues, they probably play an important role in spreading HIV infection throughout the body, although they are not themselves infected with the virus. In addition, the fact that HIV reduces CD21 levels on B-cell surfaces provides a clue as to how HIV disturbs B-cell function. In future studies, the researchers will investigate further the mechanisms by which HIV binds to CD21, which might ultimately lead to a therapy that could prevent this binding and therefore prevent B cells from spreading HIV. Also, further research on how HIV reduces CD21 levels on B cells might yield a therapy that could boost immune system function in HIV disease.

Moir S, Malaspina A, Li Y, Chun TW, Lowe T, Adelsberger J, Baseler M, Ehler LA, Liu S, Davey RTJr, Mican JAM, and Fauci AS: B cells of HIV-1-infected patients bind virions through CD21-complement interactions and transmit infectious virus to activated T cells. Journal of Experimental Medicine 192: 637-645, 2000.

Regulating the Development of the *Leishmania* Parasite's Infective Stage

Background: Parasites of the genus *Leishmania* cause several distinct diseases in humans, each of which have a range of clinical manifestations. These include cutaneous leishmaniasis, a form of leishmaniasis that causes disabling and disfiguring skin sores, and visceral leishmaniasis (also known as kala-azar), an often fatal form that attacks internal organs such as the liver and spleen. An estimated 12 million people worldwide are affected by *Leishmania* infection (leishmaniasis), and 350 million people live in areas where they may be exposed to the parasite. The type of *Leishmania* that infects humans undergoes a critical stage of development within the sandfly, the insect that transmits the parasite through its bite. Researchers had identified genes involved in the development of *Leishmania* from noninfective to highly infective forms, but the molecular mechanisms involved in the initiation and regulation of this vital step had not yet been defined.

Advance: Investigators recently identified a compound called tetrahydrobiopterin (H_4B) that regulates the development of *Leishmania* into its infective form. Specifically, they examined whether genetically altered parasites lacking an enzyme called pteridine reductase 1 (PTR1), which promotes the production of H_4B in the parasite, could infect mice. These mutant parasites, which have unusually low levels of H_4B, not only remained infectious to mice but also caused more severe disease, as measured by higher parasite numbers and increased formation of skin lesions in infected mice. This result establishes that H_4B levels in *Leishmania* play an important role in controlling the development of the parasite into its infective stage. The investigators hypothesize that by keeping H_4B levels elevated and limiting the severity of leishmaniasis in an infected individual, or "host," PTR1 fosters the survival of the host and thus increases the transmission of *Leishmania* parasites. More severe disease is more likely to kill the host, who would then be unable to continue the cycle of disease transmission.

Implications: A better understanding of the *Leishmania* life cycle, including the development of infectious forms within the sandfly, may lead to new ways to control leishmaniasis – treatments that are less toxic and more effective than the current first-line drug therapy.

Cunningham ML, Titus RG, Turco SJ, and Beverley SM: Regulation of differentiation to the infective stage of the protozoan parasite *Leishmania major* by tetrahydrobiopterin. Science 292: 285-287, 2001.

Drugs, Food, and Gambling: Is there a Commonality?

Background: Is drug addiction unique from other types of addiction? Science is now revealing that there are striking neurobiological similarities among addiction to drugs of abuse and other addictive behaviors such as gambling and pathological over-eating. In recent years, research has demonstrated that all drugs of abuse, be it cocaine, heroin, methamphetamine or nicotine, are able to increase levels of the neurotransmitter dopamine in the brain circuits that have been shown to mediate reward and reinforcement. Now, two studies using state-of-the-art neuroimaging technologies are showing that some of these same mechanisms also appear to be involved in gambling and obesity.

Advances: In a study of 12 men participating in a monetary game of chance, functional magnetic resonance imaging (fMRI) was used to map brain activity during the game. Subjects were provided with a $50 endowment and were told that they might lose some or all of the money, retain it, or increase it. The experiment was then divided into two phases, the "expectancy" phase, in which the gaming task was initiated and the "outcome" phase, in which they won, lost, or retained their money. Brain activity was mapped during these phases. Significant increases in brain activity were seen in several brain regions including the nucleus accumbens, the ventral tegmental area, amygdala, and orbitofrontal cortex in response to the expectancy and outcome phases of the game. The pattern of activity observed is strikingly similar to the responses to cocaine administered to cocaine addicts in previous studies, suggesting that these brain circuits mediate the response to diverse and highly rewarding stimuli.

In another study of 10 pathologically obese (body mass index (BMI) greater than 50) individuals, investigators found that pathological over-eating seems to be associated with reduced dopamine function. Investigators, using positron emission tomography (PET), measured the availability of the D2 dopamine receptor subtype in obese and matched control subjects. They found that the D2 availability was significantly lower in the obese subjects than for the controls. Additionally, they observed that in the obese subjects, those individuals with lowest D2 levels had the highest BMIs. Low dopamine D2 receptors have also been reported in subjects addicted to cocaine, alcohol, and opiates, suggesting that a reduction in D2 receptors is associated with addictive behavior, irrespective of whether it is due to food or addictive substances.

Implications: Eating and gambling are both highly reinforcing behaviors that induce feelings of gratification and pleasure. Neuroimaging technologies are now revealing that the brain circuits that may account for some of these behaviors are the same ones reported to be involved in drug addiction. These potential commonalities among addictions, whether it be food, gambling, or drugs, support the idea that dysfunction of brain processes crucial to decision-making and behavior may contribute to a broad range of problems.

Wang GJ, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, Netusil N, and Fowler JS: Brain dopamine and obesity. Lancet 357(9253): 354-357, 2001.

Breiter HC, Aharon I, Kahneman D, Dale A, and Shizgal P: Functional imaging of neural responses to expectancy and experience of monetary gains and losses. Neuron 30(2): 619-639, 2001.

MDMA Use During Pregnancy Can Impair Memory and Learning in Offspring

Background: 3,4-methylenedioxymethamphetamine, commonly abbreviated and referred to as MDMA or "Ecstacy," is an illegal drug that is being increasingly used by adolescents and young adults across the country. A growing body of evidence links heavy and prolonged MDMA use to confusion, depression, sleep problems, persistent elevation of anxiety, aggressive and impulsive behavior, and selective impairment of some working memory and attention processes. As the use of MDMA increases, there inevitably will be increases in the number of users who are pregnant. However, little is known about the effect of MDMA on the developing brain of an unborn child. Now, there is new evidence in animal models that MDMA may not only be harmful to the user, but potentially to offspring, too.

Advance: Researchers have found the first evidence that prenatal use of MDMA may cause memory loss and other impairments in offspring. Newborn rats were given MDMA on days 1-10 or 11-20 (time frames similar to brain and central nervous system development in humans during the early and late third trimester). The subjects ability to perform in learning and memory tasks were observed. The subjects had no difficulty learning a cued version of a swimming maze. However, when the subjects were faced with spatial learning and probe or memory trials, memory and learning deficiencies were detected in the subjects exposed to MDMA on days 11-20. The findings suggest that MDMA has selective effects on cognitive development during the CNS development cycle. Because of the way in which the study was designed, it has been determined that the results are not confounded by other factors such as stress or malnutrition. The learning deficits were found to be long-term, meaning they were seen in the offsrping as adults.

Implications: Because MDMA use continues to increase among young adults, it is critical that research findings about this drug are shared, particularly with women of child-bearing age. Many individuals still perceive MDMA as a safe, benign substance. This study confirms that users may be damaging not only their own cognitive abilities but those of their children as well.

Broening HW, Morford LL, Inman-Wood SL, Fukumura M, and Vorhees CV: 3,4-Methylenedioxymeth-amphetamine (Ecstasy)-induced learning and memory impairments depend on the age of exposure during early development. The Journal of Neuroscience 21(9): 3228-3235, 2001.

Despite Lower Initial HIV Blood Levels in Women Men and Women Develop AIDS at Similar Rates

Background: AIDS, caused by the human immunodeficiency virus (HIV), has claimed 22 million lives since the disease was recognized 20 years ago. Approximately 800,000 to 900,000 individuals in the U.S. are living with HIV infection. Fortunately, potent combinations of anti-HIV drugs (highly active antiretroviral therapy or "HAART") have dramatically reduced the numbers of new AIDS cases and AIDS deaths. However, there is still much we do not know about this disease, particularly how it progresses in the body. To learn more about progression, researchers monitored over 200 participants in the AIDS Linked to the Intravenous Experience (ALIVE) cohort. Established in 1988 the ALIVE cohort is a natural history study of HIV disease among injection drug users. Between 1988 and 1998, in one of the largest studies ever to examine gender-specific differences of HIV infection, the team followed a group of 156 men and 46 women who became HIV positive. The research team evaluated the risk of progression to AIDS by measuring several factors, including the volunteers' amount of HIV in the blood, or viral load, at regular intervals, starting from around the time they became infected with HIV and continuing for several years.

Advance: Researchers discovered that although during the first years of HIV infection women have significantly lower amounts of HIV in their blood than men, both men and women had a similar risk of developing AIDS. The team found that the median initial viral load of the 15 women who progressed to AIDS was about 4.5 times lower than that of the 29 men who progressed to AIDS. Yet, the men and women experienced a similarly swift rate of loss of their CD4+ T cells, the immune cells that decrease as a result of HIV infection. Previous studies in men show that initial viral load can be used to gauge their likelihood of progression to AIDS, but these data confirm that the initial viral load is much lower in women than in men and consequently not as predictive for women. The investigators found that the women who developed AIDS had an average initial viral load of 17,149 copies of virus per milliliter (ml) of blood versus men who progressed to AIDS whose average was 77,822 copies/ml. Even men in the group who never developed AIDS had a higher median initial viral load of 40,634 copies/ml of blood.

Implications: In addition to providing a clearer picture of the natural history of progression to AIDS, this study sheds light on the effect of gender differences on viral load and the challenges AIDS poses to women's health. The results also support recent changes in the criteria which help doctors tailor anti-HIV drug therapy to delay the onset of AIDS. According to the older criteria, the women's lower viral load levels meant that early after HIV infection a smaller percentage of them would have been eligible to start treatment with anti-HIV drugs. Despite these positive steps, more studies on the dynamics of HIV infection are still needed to determine the best time to start drug therapy, particularly for women as many new HIV/AIDS cases occur among women.

Sterling T, Vlahov D, Astemborski J, Hoover DR, Margolick JB, and Quinn TC: Initial plasma HIV-1 RNA levels and progression to AIDS in women and men. The New England Journal of Medicine 344(10): 720-725, 2001.

Key Transcription Factor May Play Role in Transition from Voluntary Cocaine Use to Addiction

Background: Identifying the cellular adaptations that occur in response to chronic drug exposure will greatly increase our understanding of the transition from drug abuse to addiction. Research is revealing that when a drug such as cocaine is taken, a number of measurable changes occur in the brain, levels of some proteins are increased, others may decrease. One potential substrate of this transition is the transcription factor, _FosB, since previous studies on mice genetically engineered to have increased levels of _FosB were found to be more sensitive to the stimulant effects of cocaine. Research is now beginning to reveal the cascade of cellular events that occur in response to chronic cocaine exposure.

Advance: Transgenic mice were produced in which researchers could experimentally increase the levels of _FosB in the brain's striatal region. The striatum is an area of the brain that has been closely linked to the reward and reinforcing effects of cocaine and other drugs of abuse. Using microarray technology, researchers found that when _FosB levels were increased, the levels of another protein, Cdk5, were subsequently increased in the striatum. To confirm Cdk5's role in mediating cocaine's effects on the brain, rats given daily injections of the Cdk5 inhibitor, roscovitine, were found to be more sensitive to the stimulant effects of cocaine than control animals. Cdk5 is known as a protein kinase, a protein that "phosphorylates" or adds phosphate groups to other cellular proteins. One way that Cdk5 could regulate the behavioral effects of chronic cocaine use is through the regulation of dopamine communication processes. Cdk5 has been shown to phosphorylate a key protein involved in dopamine communication in the striatum, DARPP-32 (dopamine and cyclic AMP-regulated phosphoprotein, 32) which in turn reduces the activity of the D1 subtype of the dopamine receptor. Several previous lines of evidence have implicated the D1 receptor in behavioral responses to cocaine.

Implications: Taken together, the data support a biochemical pathway in which repeated exposure to cocaine causes an accumulation of _FosB which in turn results in increased expression of Cdk5 resulting in phosphorylation of DARPP-32 which then reduces dopamine D1 receptor communication. This cascade of cellular events may in part contribute to the adaptive changes in the brain related to cocaine addiction. Unraveling the neuroadaptations that occur in response to chronic drug exposure will increase our understanding of what is happening in the brain during the transition from drug abuse to addiction. Understanding this transition process will be key in developing more effective treatments for drug addiction in the future.

Bibb JA, Chen J, Taylor JR, Svenningsson P, Nishi A, Snyder GL, Yan Z, Sagawa ZK, Ouimet CC, Nairn AC, Nestler EJ, and Greengard P: Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. Nature 410(6826): 376-380, 2001.

Even a Single Exposure to Cocaine Can Alter Brain Function

Background: Years of research have clearly shown that long-term drug use leads to changes in the brain, some of which might be permanent. Even though drug abuse starts out as a voluntary, preventable behavior, at some point drug use changes the brain leading to compulsive drug use even in the face of severe negative consequences. Understanding this switch from abuse to addiction will be key in helping us develop effective preventions and treatments. Research has produced many clues to the process behind this transition, but one important question remains: how much drug use is needed for that transition to take place? Research is now revealing that just one drug exposure may be enough to alter brain function.

Advance: Scientists have recently identified changes in the brain that occur with only a single exposure to cocaine. In this study, researchers studied the brains of rodents who were given either one dose of cocaine or saline as a control. They found that neural connections in a part of the brain known at the ventral tegmental area (VTA) were altered by just a single dose of cocaine. The VTA is involved in the rewarding aspects of cocaine, as well as certain aspects of learning and memory. This study also demonstrated that the changes that occurred in the VTA were still present five days after the single dose of cocaine, indicating that even a single exposure to cocaine can cause long-term changes in the brain.

Implications: These findings indicate that just one use of a drug such as cocaine may be enough to cause brain changes that, in some individuals, could trigger compulsive use – perhaps flipping the proverbial "switch" for addiction. The changes that were observed in the brain may also be important not just for understanding the early stages of addiction, but also may help explain the neural basis for relapse, where a single exposure to cocaine after a period of abstinence can induce renewed drug-seeking behavior. By understanding precisely how the brain is changed by cocaine, it may be possible to develop more sophisticated medications that target these brain changes.

Ungless MA, Whistler, JL, Malenka RC, and Bonci A: Single cocaine exposure *in vivo* induces long-term potentiation in dopamine neurons. Nature 411: 583-587, 2001.

Progress Made in Understanding the Neurobiological Basis of Relapse and the Role that Memory Plays

Background: Efforts to treat cocaine addiction are often undermined by high rates of relapse. Now, researchers have made a major advance in understanding the neurobiological basis for relapse to cocaine abuse and how it is connected to memory. It had been known previously that the learning of associations between environmental cues and taking drugs occurs in an area of the brain known as the hippocampus. These environmental cues that bring back memories of being high, such as a social situation or location, are a major cause of relapse to drug-taking behavior. Now scientists have pinpointed the region of the hippocampus and the neurobiological factors that may play an integral role in triggering drug-related memories leading to cocaine abuse relapse.

Advance: In the study, researchers first conditioned rats to obtain cocaine by pushing one of two levers which delivered a dose of cocaine. After this cocaine-seeking behavior had been well established, saline solution was substituted for cocaine. When the rats no longer received cocaine by pushing the lever, they greatly decreased their lever pushing until nearly stopping. Then, the investigators electrically stimulated a region of the hippocampus called the ventral subiculum. The ventral subiculum is involved in the memory process and is rich in the neurotransmitter glutamate. The activation of the memory circuits in the ventral subiculum led to the rats seeking cocaine by repeatedly pushing the lever that previously delivered cocaine. In contrast, electrical stimulation of the brain's dopamine rich reward center did not cause the rats to seek cocaine. Furthermore, when a pharmacological compound that blocks glutamate receptors was injected into the rats' brains electrical stimulation of the ventral subiculum did not bring about relapse. Based on these results, it appears that dopamine which produces the "rush" or reward associated with drug taking is key to drug use initiation and continued use, but that relapse is associated with the memory encoding area of the brain and the transmission of glutamate.

Implication: The findings from this study suggest that reward and relapse may function through independent pathways – reward through dopamine-regulated pathways and relapse through glutamate-regulated pathways that play a key role in the memory process. Thus, agents based on glutamate could be promising candidates as potential medications for cocaine addiction, particularly in preventing memory-associated, or environmental, cue-induced relapse.

Vorel SR, Liu X, Hayes RJ, Spector JA, and Gardner EL: Relapse to cocaine-seeking after hippocampal theta burst stimulation. Science 292: 1175-1178, 2001.

A Person's Craving for Drugs Can Override All Other Motivational Priorities

Background: Craving for cocaine is an often irresistible urge that can be triggered by environmental cues linked to past drug use, such as being with certain people or in a certain location. Previous research with imaging techniques has shown that many regions of the brain are involved in human drug craving. In many studies the arousal caused by cocaine craving was measured against neutral stimuli, such as nature scenes. This study was designed to see if the brain regions aroused in a cocaine user seeing cocaine-related stimuli, such as images of people using the drug, would be similar to regions affected in people who had never used cocaine when seeing nondrug-related evocative stimuli, such as sexually explicit images.

Advance: The researchers found that craving for cocaine is associated with the same brain circuits involved in response to other evocative nondrug stimuli. The scientists showed 4-minute films depicting drug use, nature scenes, or explicit sexual activity to cocaine users and to participants with no history of cocaine use. While watching the sex film, both users and non-users reported similar levels of arousal. Functional magnetic resonance imaging (fMRI) also confirmed similar patterns of regional brain activation in both groups. Most regions identified as cocaine craving sites were similarly activated by sexual stimuli, indicating that common circuits are involved in drug and nondrug responses. The researchers noted that in response to the sex film, drug users showed less activation in these brain sites than did the non-users, despite their reporting similar levels of arousal. This suggests that cocaine craving not only acts on the brain's reward circuits, it actually co-opts them or rearranges an individual's motivational priorities, changing the user's normal emotional responses to certain stimuli. This may have serious consequences for decision making by cocaine users. There were no differences in brain activation between users and non-users when they viewed the nature film.

Implications: These results suggest that the brain sites involved in cocaine craving are associated with emotional response, information processing, and working memory, and that cocaine craving is potentially driven by user's memory. Because cocaine and craving for the drug affect normal reward pathways and emotional circuitry, what is already known about normal learning, memory, and emotions may be applied to cue-induced craving and the development of appropriate pharmacological, behavioral, and cognitive therapies.

Garavan H, Pankiewicz J, Bloom A, Cho JK, Sperry L, Ross TJ, Salmeron BJ, Risinger R, Kelley D, and Stein EA: Cue induced cocaine craving: neuroanatomical specificity for drug users and drug stimuli. American Journal of Psychiatry 157: 1789-1798, 2000.

Structure of a Gene Expression Machine

Background: The enzyme RNA polymerase II (Pol II) is the central engine of gene expression in higher organisms. Through a process called transcription the enzyme "reads" the information encoded in the DNA, and in so doing creates an RNA message. The RNA message is then translated into protein, the class of molecules responsible for cellular activities and communications. Pol II is regulated by the activities of a number of proteins called initiation and transcription factors. If this regulation goes awry, it can impact all aspects of cellular metabolism, affecting normal development and in some cases causing cancer.

Advance: The atomic structure of Pol II from yeast was determined using x-ray crystallography on two different crystal forms. The structures of the enzyme in the two crystal forms revealed the enzyme in two different conformations, or shapes. A third 3-D structure of the same enzyme at slightly lower resolution captured it in the act of transcribing a piece of DNA into RNA. X-ray crystallographic data for these studies were collected at the Synchrotron Radiation Structural Biology Resource at Stanford University. The structure determination relied on the brilliant x-rays available at the Resource that allowed pinpointing the location of metal atoms in the structure. Based upon these novel Pol II structures, a new model for the mechanism of transcription has been proposed. The crystal structures reveal the enzyme in two states: an open form and a partly closed form. These forms differ mainly in the position of a massive region of the enzyme called the clamp, which is thought to close over the DNA as it enters the enzyme. The third crystal structure captures a freeze-frame of the transcription complex, with RNA and DNA bound to the enzyme. As the DNA enters the Pol II transcription machinery it hits a protein wall and must make a right angle turn. At the bend, the "reading" of the DNA information begins and RNA is generated. The growing RNA strand exits through a crevice as the DNA exits by a different passageway.

Implications: Although the goal of solving the enzymatic mechanism of transcription is an end in itself, it is part of a wider quest to understand the regulation of transcription. Not only do these crystal structures give cell biologists their first clear view of yeast Pol II in action, but they also open the door to seeing exactly how the enzyme interacts with the many other protein factors that regulate its activity. This may ultimately have implications for therapeutics developed for cancer and other disorders of the intricate cellular machinery, and will lead to a greater understanding of the process of normal development.

Cramer P, Bushnell DA, and Kornberg RD: Structural basis of transcription: RNA polymerase II at 2.8 Å resolution. Science 292: 1863-1876, 2001.

Gnatt AL, Cramer P, Fu J, Bushnell DA, and Kornberg RD: Structural basis of transcription: an RNA polymerase II elongation complex at 3.3 Å resolution. Science 292: 1876-1882, 2001.

Marx J: X-ray crystallography: transcription enzyme structure solved. Science 292: 411-414, 2001.

Klug A: Structural biology: a marvelous machine for making messages. Science 292: 1844-1846, 2001.

Microarray Analysis of Gene Expression in a Worm

Background: Having the complete sequence of the genome of an organism allows investigators to determine which genes are expressed in a given tissue at any time. To accomplish this, a copy of each gene sequence is bound to a glass slide as an individual spot. These slides, containing collections of hundreds of gene sequences, are called microarrays. The nematode worm, *C. elegans*, has been widely used to understand many aspects of the biochemistry and physiology of higher organisms. Although very different from a mammal in many respects, it contains many of the same genes as do humans and other mammals. Since the genome of *C. elegans* was one of the first to be sequenced, much is being learned from the analysis of gene expression of the *C. elegans* patterns using microarrays.

Advance: Investigators at Stanford University have examined gene expression patterns in the germ line of *C. elegans* using the *C. elegans* microarray resource. Unlike cells in somatic tissues such as muscle and skin of an animal, germ cells are totipotent. Thus the germline has the capability to give rise to all of the cell types of the animal and might be expected to preferentially express, among others, genes that are involved in the development of animal stem cells and the formation of gametes (eggs and sperm). In contrast, within the somatic tissues, developmental potential is more limited. In their analysis of gene expression in the germ line, the Stanford investigators found that, of the approximately 12,000 different genes tested (about 62 percent of the total number of genes) there were 1416 genes preferentially expressed in the germ line. In a later study in which approximately 18,000 genes were tested (94 percent of the total number of genes), the investigators found that 1651 genes were preferentially expressed in male animals.

Implications: Germ line cells, as well as different sexes, of the animal preferentially express certain genes. These genes can now be studied further to determine their precise role in the critical processes involved in animal stem cell development, sex-specific differentiation, and gamete formation. More generally, these studies demonstrate the feasibility of studying these complex processes on a global scale using microarrays and a model organism such as *C. elegans*.

Reinke V, Smith JE, Nance J, Wang J, Van Doren C, Begley R, Jones SJM, Davis EB, Scherer S, Ward S, and Kim SK: A global profile of germline gene expression in *C. elegans*. Molecular Cell 6: 605-616, 2000.

Jiang M, Ryu J, Kiraly M, Duke K, Reinke V, and Kim S: Genome-wide analysis of developmental and sex-regulated gene expression profiles in *Caenorhabditis elegans*. Proceedings of the National Academy of Sciences USA 98: 218-223, 2000.

Detection of Mutations in Transgenic Fish

Background: Fish may be used to assess health hazards associated with exposure to chemicals in aquatic environments and for providing alternative nonmammalian animal models in mutagenesis and carcinogenesis studies. Increasingly, fish have been embraced as valuable animal models in genetics, developmental biology, and toxicology. In some applications, such as in the assessment of health hazards associated with exposure to complex chemical mixtures or in low-dose chronic exposure regimens, fish are recognized not merely as alternatives to traditional rodent models, but as having distinct and superior benefits and providing insight into fundamental mechanisms of disease processes. Recent advances in fish transgenesis have made it possible to enhance the utility of fish by generating new animal models.

Advance: Investigators developed a transgenic fish called medaka (*Oryzia latipes*) that carries a specific gene for quantitation of spontaneous and induced mutations. In this new animal model for in vivo mutation detection, the transgenic fish carries a gene (*cII*) derived from a bacterial virus known as bacteriophage _ that easily mutates when exposed to a variety of environmental pollutants. Results from these investigators show that transgenic fish share many of the fundamental features of mutation analyses found in transgenic rodents.

Implications: This transgenic medaka is an important animal model for assessing potential health risks associated with chemical exposure in aquatic environments and for in vivo mutagenesis studies. Its small size, sensitivity, well-characterized histopathology, short generation time, and cost-effective husbandry contribute to the utility of this transgenic fish species in routine testing of a wide variety of compounds.

Winn RN, Norris MB, Brayer KJ, Torres C, and Muller SL: Detection of mutations in transgenic fish carrying a bacteriophage *λcII* transgene target. Proceedings of the National Academy of Sciences USA 97: 12655-12660, 2000.

A Promising Gene Therapy Vector

Background: The field of gene therapy has evolved from an investigative curiosity to a major focus of medical research. To date, its clinical successes have been few. There are many reasons for this lack of success in clinical studies but among the most important of these is the inadequacy of gene-delivery methods. For delivery, genes often are associated with carriers, called vectors, that are easily taken up by cells. Viruses made harmless through modification frequently are used as vectors. Virus-mediated gene transfer has focused primarily on murine leukemia virus and adenovirus and more recently on adeno-associated virus and lentivirus as delivery vehicles. But the deficiencies of these vectors have been problematic and restrict the progress of gene therapy.

Advance: Simian virus-40 (SV40) appears to be a highly promising gene delivery vehicle. An investigator at Jefferson Medical School in collaboration with scientists at the New England Regional Primate Research Center used this vector to effectively deliver genes to hematopoietic, or blood-forming, progenitor cells and to liver cells.

Implications: Although gene therapy was first studied as a potential means to treat diseases arising from gene mutations, gene delivery may be useful for a variety of ailments such as neurological diseases (Alzheimer's, Parkinson's) or endocrine dysfunctions (diabetes or other hormonal abnormalities). In order to be effective, appropriate delivery vehicles must be developed to ensure delivery of the specific genes to the correct target tissues. The SV40 vector shows promise as an appropriate gene delivery vehicle.

Jayan GC, Cordelier P, Patel C, BouHamdan M, Paul Johnson R, Lisziewicz J, Pomerantz RJ, and Strayer DS: SV-40 derived vectors provide effective transgene expression and inhibition of HIV-1 using constitutive, conditional, and pol III promoters. Gene Therapy 8(13): 1033-1042, 2001.

Strayer DS, Pomerantz RJ, Yu M, Rosenzweig M, BouHamdan M, Yurasov S, Johnson RP, and Goldstein H: Efficient gene transfer to hematopoietic progenitor cells using SV40-derived vectors. Gene Therapy 7(10): 886-895, 2000.

A Comparative Map of the Zebrafish Genome

Background: Diseases or disorders caused by faulty genes often are identified in animal models showing similar symptoms to those of patients with particular diseases. By deliberately mutating, or changing genes in animal models, scientists can characterize the apparent effects of these mutations and deduce the functions of similar genes in different animal species, including humans. Zebrafish mutations define the functions of hundreds of essential genes in the vertebrate genome. Because vertebrates share certain fundamental similarities, the identification of zebrafish mutations can provide important insight about gene functions that are conserved in other vertebrates. Numerous zebrafish mutants show characteristics that resemble human disease conditions and, in several cases, it is clear that the similar abnormalities result from inactivation of similar genes in the two species. To accelerate the molecular analysis of zebrafish mutations, there have been efforts to develop genetic maps and other genomic information for this animal. Gene maps for the molecular analysis of mutations are uniquely valuable for comparative studies. They have been used to identify groups of genes that are found on single chromosomes in zebrafish and humans. However, despite the utility of current comparative maps, additional work is required to discover the complete set of conserved single-chromosome genes and to learn the extent to which gene order has been preserved within these groups in different animal species and humans.

Advance: Investigators at the University of Oregon and Stanford University succeeded in identifying the human counterparts of 804 mapped zebrafish genes and marker molecules. Mapped comparisons revealed 139 new, conserved single-chromosome genes, in which two or more genes are on the same chromosome in zebrafish and humans. Although some conserved gene groups are large, there were changes in gene order within conserved groups. This reflected the relatively frequent occurrence of intrachromosomal rearrangements during the course of evolution.

Implications: This comparative map will accelerate the molecular analysis of zebrafish mutations and enhance the understanding of the evolution of the vertebrate genome. Ultimately, this knowledge will benefit efforts to identify human disease genes and develop therapies.

Woods IG, Kelly PD, Chu F, Ngo-Hazelett P, Yan YL, Huang H, Postlethwait JH, and Talbot WS: A comparative map of the zebrafish genome. Genome Research 10: 1903-1914, 2000.

New Methods for Analysis of Protein Phosphorylation

Background: Protein phosphorylation, in which phosphate is attached to protein molecules, is both ubiquitous and critically important in living systems. The presence of phosphate at specific sites on a protein may enhance or abolish its activity. Phosphate may be added or removed rapidly by a wide variety of specialized enzymes. These changes in phosphorylation are a central part of many complex, interconnected cellular signaling cascades and regulatory systems.

Mass spectrometric methods have been developed previously to identify and quantitate in an isolated protein the specific amino acids that are phosphorylated. These methods are now relatively routine. However, in the analysis of complex mixtures of proteins, identification of specific sites of phosphorylation, or even of phosphorylated proteins, is highly problematic. In most studies to obtain an overview of a cell's complete complement of proteins, an area of research known as proteomics, a protein is identified on the basis of a short segment of its amino acid sequence. Although this is not a serious shortcoming with respect to identification of a protein, it is highly unlikely that one would detect the particular portion of a protein that is phosphorylated. To do this requires either improved sequence coverage or specific isolation of modified peptides.

Advance: Two laboratories have approached this problem in parallel by attaching a chemical tag only to phosphorylation sites in a mixture of protein fragments called peptides or whole proteins. Labeled peptides are isolated using a chromatographic column that binds the label very tightly. Unlabeled peptides or proteins pass through the column unretained, and may be discarded or set aside for separate analysis. Labeled proteins or peptides retained on the column are then collected and identified. The critical advance in this work is the combination of specific chemical steps for attachment of a label only at sites of phosphorylation. In Dr. Aebersold's laboratory, an elaborate blocking chemistry protects reactive functional groups prior to formation of phosphate derivatives. In Dr. Chait's laboratory, researchers employed a strategy of base-catalyzed elimination of the phosphates, followed by direct derivatization of the modified amino acids.

Implications: These methods provide a means for investigation of protein phosphorylation on a system-wide basis. Coupled with previously developed isotope-coded affinity labeling chemistry, they will enable comparison of "phosphoproteomes" between cell states. The methods are not yet mature, but they are an important first step in application of proteomics to protein modifications after the proteins have been made. A complete inventory of a cell's proteins and their functions will help scientists understand how healthy and diseased cells function.

Zhou H, Watts JD, and Aebersold R: A systematic approach to the analysis of protein phosphorylation. Nature Biotechnology 19: 375-378, 2001.

Oda Y, Nagasu T, and Chait, BT: Enrichment analysis of phosphorylated proteins as a tool for probing the phosphoproteome. Nature Biotechnology 19: 379-382, 2001.

Transgenic Expression in Rhesus Monkey Placental Tissues

Background: In recent years immense progress has been made in understanding the function of individual genes through the production of mice in which genes have been added or removed. The addition of genetic information is relatively easily accomplished through injection of genetic material into the embryo. However, the situation is much more difficult with other species. Production of transgenic monkeys poses unique problems due to both practical and scientific constraints. It is thus important to try alternative strategies to produce genetically altered nonhuman primates. Included in the possible benefits of such experimentation is the possibility of modifying genes that are important in placental development. Among several alternative strategies for introducing novel genes, self-inactivating (SIN) viral vectors to deliver the gene without continuing further replication is particularly promising.

Advance: Using this approach, scientists at the Wisconsin Regional Primate Research Center, the University of Wisconsin, and the Holland Laboratory of the American Red Cross have demonstrated successful gene transfer into rhesus monkey embryos that subsequently were transferred to the wombs of three monkeys. This resulted in expression of the transferred gene in the placentas of the three animals that resulted from two pregnancies. One live animal was born from each pregnancy. The transferred gene also was expressed in amnion and umbilical cord tissue. However, the gene could not be detected in the minute amounts of tissue available from the newborn animals.

Implications: These studies demonstrate the successful integration of a gene into the products of conception in a nonhuman primate model. Also, this offers the potential for further studies that may more closely bear on human health than do the transgenic mice that have provided the majority of information relevant to mammalian gene function and regulation to date. It has special relevance to genes that are important in development and maintenance of the placenta during fetal life.

Wolfgang MJ, Eisele SG, Browne MA, Schotzko ML, Garthwaite MA, Durning M, Ramezani A, Hawley, Thomson JA, and Golos TG: Rhesus monkey placental transgene expression after lentiviral gene transfer into preimplantation embryos. Proceedings of the National Academy of Sciences 98(19): 10728-10732, 2001.

Cancer Virus Protein From Fish Induces Cancer-like Skin Lesions in Mice

Background: Tumor-inducing animal viruses have been important for elucidating the mechanisms of cell division and tumor development. Such viruses are found in a wide range of animal species including cold-blooded vertebrates. One example is the walleye dermal sarcoma, a type of cancer, occurring naturally on the skin of walleyed pike (*Stizostedion vitreum)* in locations throughout northern North America. The skin lesions change in different seasons and are not observed during the summer months. The factors that are responsible for these seasonal variations have not been determined, but they may be important for understanding the mechanisms governing the cell growth in such virally induced lesions in fish and other species. They may also shed light on basic mechanisms of cellular proliferation.

Advance: Investigators at the Ohio State University and Ohio University tested the hypothesis that a viral protein named rv-cyclin plays a central role in the development of walleye dermal sarcoma. The viral cyclin is related to cyclins found in other animals, where they are important regulators of cell proliferation. The investigators generated transgenic mice that expressed rv-cyclin of the walleye dermal sarcoma virus (WDSV) in their skin. Transgenic animals were smaller, had a reduced number of hair follicles, and females failed to lactate properly. Injury to the skin of such transgenic mice caused cancer-like changes.

Implications: Previous studies of human cyclin D1 have shown that it can be expressed in the skin of transgenic mice as well; however the accompanying abnormalities seen in this study were not observed previously. These indicate a broader role for this cyclin. This investigation also presents the first data demonstrating the potential of a cyclin to induce cancer-like skin lesions and thus identifies a new system to investigate mechanisms of tumor development caused by retroviruses.

Lairmore MD, Stanley JR, Weber SA, and Holzschu DL: Squamous epithelial proliferation induced by walleye dermal sarcoma retrovirus cyclin in transgenic mice. Proceedings of the National Academy of Sciences 97: 6114-6119, 2000.

Osteoarthritis and Effects of Estrogen Replacement Therapy on Cartilage

Background: Osteoarthritis (OA) is the most common form of joint disease and is one of the most common causes of pain and chronic disability in older adults. It is characterized by degradation and loss of cartilage accompanied by local enlargement of bone tissue. The specific mechanisms responsible for the emergence of OA in older adults are not entirely clear, but they may involve both the accumulation of damage in the affected tissues over time as well as alterations in the repair capacity of cells. Another aspect of OA is that epidemiological studies have suggested that postmenopausal women who take estrogen replacement therapy (ERT) are at lower risk of developing OA of the knee and that the protective effect is increased with the duration of ERT treatment. However, the biochemical mechanism by which estrogen can lead to a beneficial effect on joint metabolism is not known.

Advance: A team of investigators from Rush Medical College, the University of Minnesota, and Wake Forest University School of Medicine have used cynomolgus monkeys to examine the metabolism of knee cartilage to better understand development of OA in both aging animals and as a function of surgically induced menopause. In aging monkeys, the researchers found a significant decline in age-related response of cartilage cells to insulin-like growth factor 1 (IGF-1), suggesting the potential involvement of the response to this compound in age-related OA. In regard to ERT, the investigators have found that adult cartilage contains estrogen receptors and that ERT has a direct effect on specific aspects of cartilage metabolism.

Implications: Analysis of this monkey model has led to a better understanding of specific cellular events that occur in cartilage as a function of OA and in response to ERT. More broadly, these studies demonstrate the importance of nonhuman primate models for understanding aspects of human aging and women's health.

Loeser R, Shanker G, Carlson CS, Gardin JF, Shelton BJ and Sonntag WE: Reduction in the chondrocyte response to insulin-like growth factor 1 in aging and osteoarthritis. Arthritis and Rheumatism 43: 2110-2120, 2000.

Richmond RS, Carlson CS, Register RC, Shanker G, and Loeser RF: Functional estrogen receptors in adult articular cartilage. Arthritis and Rheumatism 43: 2081-2090, 2000.

Adolescent Suicide: Identifying Risks and Protectors

Background: Between 1979 and 1997, the overall death rate for suicide among 10- to 14-year-olds doubled; it increased by 13 percent among 15- to 19-year-olds. This made suicide the third leading cause of death among 10- to 19-year-olds in the U.S. by 1997. During this time period, the greatest increase in suicide rates was among black and other minority youth. To better understand the factors contributing to these dramatic increases, NIH scientists identified specific risks and protective factors for suicide attempts among black, Hispanic, and white male and female adolescents.

Advance: NIH researchers, in collaboration with the National Center for Injury Prevention and Control, used data from the 1995-1996 National Longitudinal Study of Adolescent Health to examine factors at the individual, family, and community level that predicted or protected against suicide attempts. A nationally representative sample of 13,110 students in grades 7 through 12 completed two in-home interviews, an average of 11 months apart.

Findings from the national survey indicate that feeling close to one's parents or family, feeling that one's parents care about them, or feeling loved and wanted by family members helped to protect against suicide attempts for black, Hispanic, and white girls and boys. For girls, emotional well-being was also "protective" for all of the racial/ethnic groups studied, while a high grade-point average was an additional protective factor for all of the boys. For both girls and boys, cross-cutting *risk* factors included previous suicide attempts, being a perpetrator or victim of violence, using alcohol and marijuana, and having other school problems such as trouble paying attention and getting homework done. Additionally, for all girls, having somatic symptoms (e.g., headache, stomachache, fatigue, and weakness), being aware of a friend's suicide or suicide attempt, using other illicit drugs, and having a history of mental health treatment predicted suicide attempts. Weapon-carrying at school and same-sex romantic attraction predicted suicide attempts for all groups of boys. Estimating the probabilities of attempting suicide for adolescents who had increasing numbers of risk and protective factors revealed that having at least 3 protective factors reduced the risk of a suicide attempt by 70 to 85 percent for each of the gender and racial/ethnic groups studied, no matter what or how many risk factors were identified.

Implications: In these national samples of black, Hispanic, and white youth, researchers identified unique and cross-cutting factors, for both girls and boys, that predict or appear to protect against suicide attempts. By identifying critical groupings and a minimum threshold of protective factors, the researchers provided a new framework for designing and improving interventions to reverse current suicide trends. This is particularly important for a growing number of minority youth.

Borowsky IW, Ireland M, and Resnick M: Adolescent suicide attempts: risks and protectors. Pediatrics 107: 485-493, 2001.

Basic Fibroblast Growth Factor Stimulates Continued Growth of New Nerve Cells

Background: A central fact of brain biology is that neurons, or nerve cells, are in a stage of terminal differentiation. This means that neighboring nerve cells cannot divide and replace those nerve cells that are destroyed or injured from congenital, inherited, vascular, or degenerative brain diseases or other conditions. Until recently, the search for factors that might encourage new nerve cell growth has been unsuccessful.

Advance: The granule nerve cell population in the cerebellum, or hindbrain, in the newborn rat model is the main focus of this study. Unlike the majority of nerve cell populations, the granule nerve cell population is formed mostly during the postnatal development period, the first three weeks after birth. A growth factor called basic fibroblast growth factor (bFGF) is known to regulate nerve cells in the developing cortex, or outer layer of the brain. Previous findings have shown that bFGF stimulated precursor nerve cells divide in newborn rats. NIH scientists have now demonstrated that a single injection of bFGF into a newborn rat significantly increases the number of nerve cells in the hindbrain. Specifically, after injecting bFGF, the number of nerve cell divisions doubled after a 72 hour period, resulting in a large increase in nerve cells and in a 22 percent increase in brain volume. Moreover, the increased number of nerve cells was sustained for two weeks after the postnatal development period. This finding shows that a single injection of bFGF can induce nerve cell growth that persists beyond the postnatal development period.

Implications: The finding that a single injection of bFGF can increase and sustain nerve cell growth in an animal model is of major clinical importance. Based on this advance, scientists may be able to translate this method of stimulating nerve cell growth into a greatly improved way to treat congenital, degenerative, or vascular diseases of the brain in humans. In addition, one of the current challenges of treating neural conditions, especially in the brain, is to find treatments that provide long-term benefits beyond temporary remedies that require patients to continually return for treatment. This study shows that bFGF may be a promising way to overcome this challenge.

Cheng Y, Tao Y, Black IB, and DiCicco-Bloom E: A single peripheral injection of basic fibroblast growth factor (bFGF) stimulates granule cell production and increases cerebellar growth in newborn rats. Journal of Neurobiology 46: 220-229, 2001.

Brain Plasticity and the Very Early Perception of Speech

Background: We assume that most children will automatically learn to talk in the first years of life, with seemingly little effort on our part or theirs. There have been two major competing theories of how children develop language – nature versus nurture. That is, children are either born with basic language ability that simply emerges as they are exposed to language, or they must learn language through the nurturing and teaching from parents, other adults, and other children. More recently, scientists have realized that both concepts are probably true and are now trying to determine how a child learns language based on both theories. Using some clever techniques to gauge what children perceive or understand, scientists are studying the language acquisition process from quite early in infancy.

To date, NIH-supported scientists have shown that, in the earliest periods of development, infants show a rich ability for learning language. Studies across various languages show that infants have an extraordinary ability to learn the unique properties of the language spoken around them. The scientists are now studying infants' perception of speech between ages 6-12 months, using brain imaging techniques. The scientists are also analyzing reactions to the language an infant hears to determine how "plastic," or flexible, the infant's language systems are when they hear a foreign language, and how the language may actually change the infant's perception and brain. Using these newest methods, scientists continue to study the brain-biology connection in children's development of language.

Advance: The scientists studied infants between 6-8 months and between 10-12 months of age who were exposed to American English and either to Japanese or to Taiwanese. There are certain sounds that have meaningful differences in English but do not apply in either Japanese or Taiwanese. For example, in English the "r" and "l" sounds contrast in words, but Japanese speakers have difficulty making these sounds because, in their language, these sounds act as one sound or phoneme. The scientists found that between ages 6-8 months, infants do not differ in their responses to such sounds, no matter which language their parents speak. However, when the infants are between ages 10-12 months, they show different responses. This reflects the fact that the infants seem much more familiar with sounds that have meaningful differences in the languages their parents use, while the infants' ability to recognize the non-meaningful sounds declines. In another study, the scientists found that infants who were exposed to foreign languages between ages 6-8 months continued to recognize the sounds of these languages; thus, at this early age infants are not yet limited to the specific sounds of one language.

Implications: This research on how infants perceive the sounds of various languages has significant implications for theories about whether there are critical learning periods for certain types of information, brain plasticity, and the complex interaction of biology and culture. Furthermore, these findings may help parents who struggle with the best way to prepare their children to learn languages early in life. This is especially important as many children grow up in communities where their first language is not English, and acquiring these skills is crucial to their education and well-being.

Kuhl PK: A new view of language acquisition. Proceedings of the National Academy of Sciences USA 97: 11850-11857, 2000.

Bullying Among Middle and High School Youth

Background: Bullying among school-aged youth is increasingly being recognized as an important problem affecting their well-being and social functioning. While a certain amount of conflict and harassment is typical of peer relations among youth, bullying presents a potentially more serious threat to healthy development. Bullying is defined as a specific type of aggression in which 1) the behavior is intended to harm or disturb, 2) the behavior occurs repeatedly over time, and 3) there is an imbalance of power, with a more powerful person or group attacking a less powerful one. While bullying among youth is frequently reported as a concern by parents, educators, and youth themselves, little research examining bullying has been conducted among U.S. youth. International studies have shown a wide range of reported prevalence of bullying and have linked bullying to poorer social and emotional functioning. However, prior to studies by NIH researchers, no information on the national prevalence of bullying was available.

Advance: NIH researchers, in collaboration with the World Health Organization, conducted a nationally representative survey on the health behavior of U.S. school children, including bullying. In addition, research on bullying in one Maryland county was conducted as part of a study of a middle school problem behavior prevention program.

Significant findings from the national survey indicate that 30 percent of youth in grades six to ten report involvement in moderate or frequent bullying: 13 percent have bullied others, 11 percent have been bullied, and 6 percent have both bullied others and been bullied. In both studies, bullying and being bullied were associated with poorer social, emotional, and behavioral functioning. Researchers found that those who bullied others showed greater behavior problems including alcohol use, smoking, and poorer school achievement. Youth who have been bullied were more likely to be socially isolated and depressed. Both bullies and those bullied showed poorer school adjustment, social competence, and self-control, as well as less parental involvement and guidance.

Implications: This study provides the first national prevalence data of bullying among U.S. youth and identifies potential risk and protective factors. Given the current behavioral and emotional difficulties associated with bullying, as well as the potential long-term negative health outcomes for bullying and bullied youth, the issue merits serious attention in terms of future research and in terms of developing effective preventive interventions. This study provides the critical research foundations needed to begin these efforts.

Nansel TR, Overpeck M, Pilla RS, Ruan WJ, Simons-Morton B, and Scheidt P: Bullying behaviors among U.S. youth: prevalence and association with psychosocial adjustment. The Journal of the American Medical Association 285: 2094-2100, 2001.

Haynie DL, Nansel TR, Eitel P, Crump AD, Saylor K, Yu K, and Simons-Morton B: Bullies, victims, and bully-victims: distinct groups of youth at risk. Journal of Early Adolescence 21: 29-49, 2001.

First National Data to Understand Where Children Drown

Background: Drowning is the second leading cause of unintentional injury death among children aged 1 to 19 in the U.S. Determining the details about specific sites where drowning occurs is important for improving prevention programs. However, such national data have been unavailable. To address this lack of information, NIH scientists collected national data about the specific sites where children drown in the U.S. Specifically, the scientists collected information on children who were under age 20 and who died by unintentional drowning. The scientists grouped specific drowning sites into four categories: artificial pools (swimming pools and hot tubs), freshwater bodies (lakes, ponds, rivers, canals, and other specified sites), domestic sites (primarily bathtubs and buckets), and salt water. Then, the scientists examined the site-specific drowning rates by age, race, and gender.

Advance: The scientists found that infants are most likely to drown in bathtubs, toddlers in swimming pools, and older children in other freshwater sites such as rivers and lakes. Of all the children who drowned, nearly 75 percent of them were male. Consistent with previous studies, the scientists also found that toddlers and adolescent males had increased risks of drowning, and, among adolescent males, rates were higher for African-Americans than for whites. The scientists found that after age 5, African-American males were up to 12-15 times more likely to drown in swimming pools than white males. After age 5 for females, drowning rates were low, but African-American females were also at increased risk of drowning in a swimming pool compared to white females of the same age.

Implications: The NIH scientists are the first to examine and present national data on where U.S. children tend to drown. Each year, about 1,500 children drown in the U.S. These data confirm that a disproportionate number are African-American children, which is consistent with findings from small regional studies. Since many of these drowning deaths may be preventable, this comprehensive look at the data establishes the critical foundation for determining the underlying factors that contribute to this racial disparity, and the basis for developing targeted interventions to prevent swimming pool drownings among African-American youth.

Brenner RA, Trumble AC, Smith GS, Kessler EP, and Overpeck MD: Where children drown, United States, 1995. Pediatrics 108: 85-89, 2001.

New Clues Regarding the Role of Bacteria Infections in Premature Births

Background: The fluid-filled sac that surrounds a developing fetus is made up of fetal membranes, amnion and chorion, which are connective tissue structures. Sometimes these fetal membranes break several weeks before the end of the gestation period leading to premature labor. This condition is known as premature rupture of membranes (PROM) which is one of the most common causes of premature birth in the U.S. and accounts for approximately 40,000 premature births each year. NIH scientists discovered that more than one-third of the mothers who experience PROM have bacteria present within the amniotic sac. Until recently, scientists did not understand what factors were responsible for PROM or how PROM was associated with infections of the uterus. Specifically, scientists were uncertain whether enzymes produced by the bacteria directly caused the membranes to rupture, or whether the membranes ruptured in response to a maternal or fetal immune response to an infection. To answer these questions, the scientists studied a family of enzymes, matrix metalloproteinases (MMPs), which were identified as potentially playing a key role in PROM. MMPs break down collagens and collagens are needed to hold the fetal membranes together. The researchers hypothesized that infections of the uterus somehow cause changes in MMP levels and that these changes weaken the fetal membranes, leading to membrane rupture and premature labor.

Advance: Using newly devised techniques to measure MMP levels in the amniotic fluid, scientists discovered that certain MMP levels rise while others fall in association with PROM and with spontaneous labor. Scientists observed the same pattern of rise or fall in MMP levels when an infection is present within the amniotic sac. The enzymes break down the collagens in the fetal membranes, leading to PROM, which in turn may cause spontaneous labor. Furthermore, scientists identified that the MMPs are produced by both the uterus and the fetus, not by the bacteria. While it has been suggested that giving antibiotics to women with amniotic infections may prevent PROM and premature labor, the same researchers showed that antibiotic therapy does not work. Indeed, in some cases antibiotics make the situation worse, because killed bacteria sometimes release toxic substances.

Implications: These observations strongly support the theory that MMPs play a critical role in events leading to PROM and premature labor. The findings further support the notion that PROM is the consequence of an immune response to infections of the uterus in some women and their infants. Why some women and infants develop a more severe response is still unknown, but is believed to be caused by genetic factors. Scientists can now work to identify the genetic factors that predispose some women to PROM, and to discover new ways of preventing the heightened immune response and, ultimately, to prevent PROM and premature labor.

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Maymon E, Romero R, Pacora P, Gervasi MT, Gomez R, Edwin SS, and Yoon BH: Evidence of in vivo differential bioavailability of the active forms of matrix metalloproteinase 9 and 2 in parturition, spontaneous rupture of membranes, and intra-amniotic infection. American Journal of Obstetrics and Gynecology 183: 887-893, 2000.

Old Drug Offers New Hope to Victims of Childhood Neuro-Degenerative Disease

Background: Batten disease represents a group of fatal genetic disorders of the nervous system that begins in early childhood. Infantile neuronal ceroid lipofuscinosis (INCL) is a rare form of Batten disease, which claims the lives of its victims at a much earlier age than other forms of the disease. Also called Santavuori-Haltia disease, INCL strikes children between 6 months and 2 years of age. Children who have INCL lose their eyesight by age 2, experience frequent seizures, and deteriorate mentally until their brain has no cortical activity at 3 to 4 years of age. They live in this vegetative state until they reach about 8 to 12 years of age, when they die. INCL is caused by a defect in the gene that instructs the formation of palmitoyl-protein thioesterase (PPT). PPT is needed to break bonds that hold certain protein-lipid compounds together. These protein-lipid molecules are byproducts of normal chemical reactions in the cell. They are stored in lysosomes, microscopic sacs that are the cell's garbage disposal units, until they can be disconnected and transported to other parts of the cell for recycling. Because children with INCL lack PPT, the protein-lipid compounds accumulate within their lysosomes. Eventually, the lysosomes grow so large that they kill the cell.

Advance: NIH researchers used cell cultures taken from patients with INCL to test two commercially available drugs, phosphocysteamine and N-acetylcysteine, that potentially could break the protein-lipid bonds. The researchers found that, in laboratory conditions, both drugs successfully reduced the amount of protein-lipid compounds within the lysosomes and prevented the compounds from reaching toxic levels. Of the two drugs tested, phosphocysteamine has an additional advantage in that it can enter brain cells and pass through the placenta. Given these promising findings, a clinical trial has been approved for patients with the most severe form of INCL to test whether phosphocysteamine will be effective in treating infants and young children. The first patient for the study has been enrolled and is now receiving the drug treatment.

Implications: The NIH research team demonstrated in laboratory studies that phosphocysteamine shows particular promise for treating INCL. This is especially encouraging because phosphocysteamine is a relatively nontoxic drug that has been used safely for twenty years to treat another rare genetic disease, cystinosis. Furthermore, because the drug can cross the placenta and enter the brain, it has potential as both a prenatal and postnatal treatment.

Zhang Z, Butler JD, Levin SW, Wisniewski KE, Brooks SS, and Mukherjee AB: Lysosomal ceroid depletion by drugs: therapeutic implications for hereditary neurodegenerative disease in children. Nature Medicine 7: 478-484, 2001

New Evidence Shows Risk for Adult Hypertension and Cardiovascular Disease Begins Before Birth

Background: The origin of adult disease stems from many factors, including genetics and "lifestyle" or environmental factors. One intriguing hypothesis offered nearly a decade ago by Dr. David Barker proposes that events influencing the environment within the uterus predispose a fetus to develop disorders years later. For example, poor nutrition, maternal diabetes, preeclampsia, a high-stress environment, or certain medications may affect the fetus in ways not yet understood, leading to future health problems. Since then, a number of epidemiological studies have also suggested that factors in the early uterine environment result in the onset of major adult diseases, such as diabetes, obesity, hypertension, and cardiovascular disease. Although the Barker hypothesis remains controversial, accumulating research provides increasingly strong support for it, and suggests that perinatal factors, such as maternal nutrition, can "program" the fetus for increased disease risk in later life. However, the physiologic and molecular mechanisms that cause this programming remain unknown.

Advance: An NIH-supported researcher has demonstrated that moderately restricting the protein in the diets of pregnant rats results in pups born with reduced weight and impaired kidney development. This impairment persists into adulthood and results in decreased kidney function and increased pressure in the arteries, leading to hypertension and an increased risk for developing cardiovascular disease. The researcher has also shown that this impairment occurs due to the suppression of the kidney's renin-angiotensin system (RAS) during fetal life. The RAS is a hormonal system known to be important in regulating blood pressure and blood volume in adults.

Implications: Based on these findings, it is likely that the RAS plays other critical roles during fetal development, including establishing the total number of nephrons (tiny filtering devices) in the kidney and programming an individual's normal blood pressure level in adult life. These findings point to a potential pathway in the fetal environment that can "program" an individual for hypertension and an increased cardiovascular risk later in life. Understanding such pathways is the first step towards conceptualizing and eventually developing interventions to help prevent these conditions.

Woods LL, Ingelfinger JR, Nyengaard JR, and Rasch R: Maternal protein restriction suppresses the newborn rennin-angiotensin system and programs adult hypertension in rats. Pediatric Research 49: 1-8, 2001.

Woods LL: Fetal origins of adult hypertension: a renal mechanism? Current Opinion in Nephrology and Hypertension 9: 419-425, 2000.

Activin Receptors – A New Anticancer Signal in Human Tumors

Background: The major problem with human tumors is a social one – tumor cells do not obey the signals from their surrounding cells that should restrain their growth. To date, very few of these signals have been defined, which limits our ability to understand and oppose this basic abnormality. Because of the need to understand these signals, there has been great effort to identify genes that are mutated and turned off, or inactivated, in tumors. The inactivation of these tumor-suppressor genes allows tumors to avoid the normal environmental growth controls.

Advance: Mutations within the activin receptor gene were found in some pancreatic cancers in the past year, and similar mutations are being sought in other cancer types. Activin is a protein that is secreted by normal cells. To exert its action, activin must bind receptors on a cell. The receptors send a signal to the cell, but it was not previously known that these signals were able to suppress tumor growth. In tumors that lack mutations in the activin receptor gene, it might be possible to administer activin as a treatment strategy or to mimic the effects of activin on tumor cells by precisely targeting the signaling pathway itself.

Implications: Researchers are exploring this new idea to try to discover a therapy that could specifically attack the most vulnerable components of human tumors.

Su GH, Bansal R, Montgomery E, Yeo, CJ, Hruban RH, and Kern SE: ACVR1B (ALK4) gene mutations in pancreatic carcinoma. Proceedings of the National Academy of Sciences USA 98: 3254-3257, 2001.

Silencing of Apoptosis Genes in Childhood Brain Tumors

Background: Neuroblastoma is the most common extracranial solid tumor occurring in children. Despite major advances in cancer chemotherapy and the use of bone marrow transplantation, the long-term survival rate for patients with aggressive forms of the disease remains very low. One of the reasons for the poor prognosis associated with neuroblastoma has to do with its resistance to chemotherapeutic agents that work by inducing apoptosis, or programmed cell death. Apoptosis is a normal biological process whereby damaged or unneeded cells undergo a series of self-induced events that lead to their destruction and absorption by the body. In the absence of this essential means of self-elimination, cancer cells are able to rapidly multiply out of control, to the detriment of surrounding healthy tissue. Because the suppression of apoptosis is known to be a major characteristic of neuroblastoma cells, investigators have been searching for clues as to how apoptosis is prevented in these cancer cells.

Advance: NIH-supported researchers examined 18 human neuroblastoma cell lines and found that in 13, the gene responsible for producing caspase 8, an enzyme that is instrumental in inducing apoptosis, was inactivated. The inactivation of this gene occurred almost exclusively in neuroblastoma cell lines in which the *MYCN* oncogene was amplified (i.e., there were extra copies of the gene). Neuroblastoma cells with *MYCN* amplification are among those with a particularly poor prognosis and are often resistant to irradiation or chemotherapy. The lack of caspase 8 in these cells seemed to protect the cancer cells against a number of mechanisms that effect cell death, including, most importantly, chemotherapeutic agents that induce apoptosis in cancer cells by damaging their DNA. This resistance to apoptosis was reversed when caspase 8 was re-introduced into neuroblastoma cells lacking the enzyme.

Implications: The investigators found that the mechanism responsible for silencing the expression of caspase 8 in many of the neuroblastoma lines was DNA methylation (a process by which CH_3 groups are added) of the gene. This finding raises the possibility that demethylating agents may be effective in the treatment of neuroblastoma, particularly those types with *MYCN* amplification. Repair of a cancer cell's apoptotic machinery offers the promise of effective therapy for neuroblastoma.

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Cell Phone Use Does Not Increase Risk of Brain Tumors

Background: Hand-held cellular telephones became available to U.S. consumers in 1984, and by the year 2000, 92 million Americans had one. Hand-held cell phones have antennas that radiate low-power microwave frequency signals. Cell phone users put this source of radio frequency radiation next to their heads, and a growing number of news reports raised concerns that this might cause brain tumors or contribute to their growth. With 500 million cell-phone users worldwide, the question had important public health implications. And because no one knew whether cell phone radiation was a cancer risk, in 1993 NIH researchers included cell-phone use as part of a comprehensive study on adult brain tumor causes. The ongoing study involves 800 adults with brain tumors and 800 controls – people who do not have brain tumors – from three medical institutions in Phoenix, Boston, and Pittsburgh. Data was collected until 1998 through personal interviews, where participants were asked when they first used a hand-held cell phone, the date of last use, and how often they used a cell phone.

Advance: NIH researchers found no evidence that people who use hand-held cell phones have a greater risk of developing tumors of the brain or nervous system compared to nonusers. The researchers found no evidence that a person's risk of developing a brain tumor increased with increasing years of use or average minutes of use per day. And brain tumors among cell-phone users did not occur more often than expected on the side of the head on which the person reported using the phone.

Implications: The NIH study began in 1993 and ended in 1998, during a time when most people used analog (800-900 MHz) cell phones. Today most people use digital cell phones, which operate at a lower average power than analog phones. No increase in the risk of brain tumors associated with analog or digital cell phones was found in a small study conducted in Sweden, and there is no evidence now that cancer risk would differ for analog or digital cell phones.

Inskip PD, Tarone RE, Hatch EE, Wilcosky TC, Shapiro WR, Selker RG, Fin HA, Black PM, Loeffler JS, and Linet MS: Cellular-telephone use and brain tumors. The New England Journal of Medicine 344: 79-86, 2001.

Identifying Gliomas That Will Respond to Chemotherapy

Background: Gliomas are common malignant brain tumors that are usually fatal in a short time, even among patients who receive treatment. But one major subtype of glioma, oligodendroglioma, is remarkably sensitive to chemotherapy; about two-thirds of patients with oligodendrogliomas treated with the PCV regimen – a combination of the drugs procarbazine, lomustine and vincristine – have long-term remissions. Until recently there was no way to tell in advance which oligodendroglioma patients would respond in this way to chemotherapy. This interferes with advising treatment courses for oligodendroglioma patients, and causes some patients who receive PCV to have unrealistic expectations of therapy.

Advance: Researchers have discovered several genetic alterations that predict response to PCV. An earlier study indicated that a loss of a portion of chromosome 1 in oligodendroglioma cells predicts response to PCV; this finding has since been confirmed. In a separate study, investigators found that patients whose tumor cells display loss of portions of both chromosomes 1 and 19, in the absence of other genetic abnormalities, are more likely to respond to chemotherapy and to live longer. Patients whose tumors have intact chromosomes 1 and 19 but have abnormalities in other genes tend to have weaker, briefer responses to chemotherapy and shorter survival, while those without alterations of the p53 gene have a particularly poor prognosis.

Implications: This discovery makes it possible for doctors to recommend the most appropriate treatment for oligodendroglioma patients. Patients whose tumors are unlikely to respond to PCV treatment will not have to go through this potentially toxic chemotherapy, and may be offered alternative treatments.

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How Cells Sense Oxygen

Background: How cells sense changes in oxygen is a fundamental question in cell biology. Chronic lack of oxygen is associated with the ability to grow blood vessels and, thus, with tumor progression.

In the cells of mammals, the lack of oxygen, or hypoxia, stabilizes hypoxia-inducible factor, or HIF, which manufactures RNA from DNA. HIF's targets include genes that play critical roles in blood vessel growth and glucose metabolism. When the cell does not lack oxygen, the E3 protein containing a specific tumor-suppressor protein tries to break down the HIFα subunit of HIF.

Advance: Researchers have identified the mammalian oxygen sensor that regulates the activity of HIF. This sensor is an intracellar enzyme that attaches a hydroxide group at HIFα amino acid 564 in the presence of oxygen and iron.

Implications: Depending on the specificity of this sensor, it may be possible to develop drugs that target the oxygen-sensing and -signaling pathways that modulate HIF in response to the lack of oxygen. These drugs may be effective in treating diseases such as stroke, heart attack, and solid tumors.

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Silicone Breast Implants Not Linked to Most Cancers or Increased Mortality

Background: An estimated 1.5 million to 2 million U.S. women have had breast implants since these devices first appeared on the market in 1962. Although silicone was initially assumed to be biologically inactive and to have no harmful effects, several short-term studies and anecdotal reports suggested that women with breast implants may have increased risks for tumors that develop from connective tissue. Of particular concern has been a possible increased risk for multiple myeloma, because a laboratory study reported that mice injected with silicone gel developed tumors derived from the same kind of cells from which multiple myeloma develops. Previous studies also reported that the risk for cervical and vulvar cancers among women with breast implants was higher than that of the general population. To address many of the unanswered questions, Congress directed the NIH to undertake a large follow-up study to assess the long-term health effects of exposure to silicone breast implants.

Advance: NIH researchers found that women with implants, as compared with a control group, were not at significant risk for most causes of death or for most cancers. Study participants were from 18 plastic surgery practices and included 13,500 women who had implant surgery for cosmetic reasons and a comparison group of 4,000 women of similar ages who had some other type of cosmetic surgery (e.g., removal of fat from the stomach or wrinkles from the face or neck). The average length of follow-up was 13 years. The only cancers for which researchers found an increased risk among women with implants were cancers of the lung, larynx, and brain. The rates of these cancers were between two to three times greater among women with implants than among those who had undergone other types of cosmetic surgery. Of these increased risks, only the rates of respiratory cancers reached statistical significance. The reasons for the higher risks observed for respiratory and brain cancers are not clear, but it is possible that they are not related to silicone exposure but are either chance findings or due to factors common to women who choose to have implants. Compared with the general population, women with implants had decreased risks for nearly every cause of death, including all cancers; circulatory and digestive system diseases; endocrine, nutritional, metabolic and immune diseases; and cirrhosis of the liver. The implant patients did not have higher mortalities from diseases that have been tentatively linked to silicone exposure.

Implications: Whereas previous reports have limited their analyses to mortality from breast cancer, this study is the first to look at all causes of mortality for women with breast implants. The lower mortalities of the implant population support previous findings that people who undergo elective surgery are generally healthier than their peers in the general population. The lower rates are due primarily to fewer deaths from cancers and diseases of the circulatory system, the most common causes of death in the general population. Further analyses of the data will evaluate the risks associated with various causes of mortality and the risk of developing connective tissue disorders

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Newly Discovered Gene for Juvenile Polyposis May Lead to Better Cancer Prevention and Clues about What Triggers Other Gastrointestinal Cancers

Background: Juvenile polyposis is a genetic disease in which people, beginning while they are still children, form growths in the digestive tract areas such as the stomach, small intestine, and colon. The polyps can cause bleeding and damage in the intestine, and afflicted individuals run a 50 percent chance of developing gastrointestinal tract cancer. People with the condition must have repeated inspections of the gastrointestinal tract with an endoscope in order to identify polyps for surgical removal, and the surgery that can control the condition usually involves removal of portions of the colon or other parts of the gastrointestinal tract. In earlier work, scientists identified mutations of a gene that caused juvenile polyposis in about 20 percent of the patients. A primary goal of researchers has been to find the genetic component that caused disease in the other 80 percent.

Advance: Researchers have found a gene affecting other juvenile polyposis patients. The scientists looked at the genes in tumors of four families who did not have the gene mutations already identified. Analysis of a suspicious chromosome area established that the gene *BMPR1A* might be critical. (Because of its original discovery in bone formation, BMP stands for "bone morphogenesis protein." In fact, it has many functions, including the regulation of cell division.) The *BMPR1A* gene produces a receptor, BMPR1A, that is widely distributed throughout the body. The receptor provides a sort of gate through the wall of an individual cell through which the BMP protein can affect the actions of the cell's DNA. In their investigation, researchers found that people who had juvenile polyposis had mutations of gene *BMPR1A*, and those that did not have polyposis tended to have no mutation. These mutations created "stop codons" which would halt the production of the BMPR1A receptors. Without the receptors, BMP could not regulate the cell division. The discovery of the mutations, and their effects on people, provided compelling evidence that this gene is responsible for juvenile polyposis in the families studied. Also, this research provides the first genetic evidence that BMPs may play a key role in controlling some cancers.

Implications: With the knowledge of this gene, patients who are at risk for juvenile polyposis but do not yet have symptoms can be screened to establish whether or not they have these mutations. Patients who do not have them can be spared expensive and repetitive screening colonoscopies and upper endoscopies. Patients with the mutations can be followed closely to keep them from developing cancer. In addition, research on the BMP pathway may produce new information about how it affects other cancers.

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Molecular Profiles Identify Genetically Distinct Subsets of Cancerous Tumors

Background: All cells have unique "signatures" – special characteristics such as which genes are active and which proteins or other products the cell manufactures. New technologies are enabling scientists to read and understand these unique cellular signatures. During the transformation of a normal cell to a cancer cell, the cell's signature changes, and that change becomes a signal of the presence of cancer. Powerful new "molecular profiling" technology, which can identify the unique molecular signatures of cancer cells, holds enormous promise for improving the early detection, diagnosis, and treatment of cancer. This technology is now being used to identify genetically distinct subsets of tumors that have different clinical courses.

Advance: The research by two groups of NIH-supported investigators has demonstrated the potential of molecular profiling for tumor classification, and provided insights into two different tumors. In an advance reported last year, scientists at NIH and the University of Nebraska have shown that diffuse large B-cell lymphomas – a diverse group of tumors with variable clinical course – comprise two distinct subsets of tumors that arise from B cells at different stages of differentiation. Patients with a tumor subtype known as "germinal-center B-cell–like" lymphoma had significantly better outcomes than those with another subtype, known as "activated B-cell–like" lymphoma. Now investigators at Stanford University have identified two genetically distinct subsets of breast tumors that appear to derive from different types of cells in the breast. One tumor subset is estrogen-receptor–positive; the other subset is estrogen-receptor–negative. It is known that patients with estrogen-receptor–positive breast tumors generally have better outcomes than patients whose tumors are estrogen-receptor–negative. These findings suggest that, by identifying the molecular characteristics of patients' tumors, it may be possible to distinguish those patients who would benefit from early, aggressive therapy from those who can be spared further therapy.

Implications: Molecular profiling has identified previously unsuspected biological features of tumors from two sites. These insights hold the promise of changing how clinical decisions are made for individual patients. Classification of patients based on molecular profiles will help oncologists select the most effective therapy for an individual and may help in the development of therapies that are targeted to specific subsets of tumors.

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Medullary Pancreatic Cancer: A New Classification for an Old Disease

Background: Human cancers are not all alike, which means that prevention and treatment of them can vary. For example, two cancers may appear to be the same, but one will respond to surgery, chemotherapy, or radiation, while the other is resistant. Over the past century, scientists have explained many of these differences by classifying cancers by their subtle differences, such as which tissues they originate from, the appearances of their cells, slight genetic changes in the DNA, or differences in their cell contents.

Advance: Scientists have newly classified a "medullary cancer" that may represent 6 percent of all pancreatic cancers and affect thousands of people each year in the U.S. (The medulla of any organ is the soft, marrow-like part of the organ.) When scientists compared the cellular characteristics of medullary cancer and other pancreatic cancers, they found a number of significant differences. For example, the genetic profile of medullary cancer is different from that of other pancreatic cancers, meaning that patients can be diagnosed at the molecular level and may respond differently to treatment. In addition, the researchers found that persons with medullary pancreatic cancer often have close relatives with breast, lung, colon, and other cancers, and may themselves be more susceptible to cancer of the colon and rectum and thus be candidates for genetic counselling and aggressive screening. Medullary cancers do not have the genetic profile of other, recently established, familial cancers, but may be a key clinical clue to the presence of an inherited cancer syndrome.

Implications: If pathologists can distinguish medullary cancer from other pancreatic cancers, scientists will be able to establish if these cancers have a different way of growing and affecting people. Further, by studying the genetics of this cancer and how it has affected whole families, they may be able to uncover new information on inherited cancers. They may even be able to find the underlying gene defect for a whole range of cancers. The new information from this classification will enable doctors to genetically counsel patients more accurately and perhaps prevent future cancers.

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New Findings on *BRCA2* Mutations and Hereditary Ovarian Cancer

Background: Mutations in the genes *BRCA1* and *BRCA2* account for a predisposition to cancer in most families with the breast-ovarian cancer syndrome. In this syndrome, the probability of finding a mutation in a woman with ovarian cancer increases with the number of related cases of ovarian or early-onset breast cancer in her family. Researchers do not know yet what proportions of ovarian cancer in the general population come from *BRCA1* and *BRCA2* mutations, or whether cases of ovarian cancer associated with *BRCA2* mutations are different from those associated with *BRCA1* mutations.

Advance: To answer these and other questions, NIH-supported researchers studied 649 cases of ovarian cancer from the Ontario (Canada) Cancer Registry that had been diagnosed in 1995-96. Investigators screened for 11 of the most common mutations, seven in BRCA1, four in BRCA2. The researchers found no mutations in 134 women with noninvasive tumors; they identified 60 mutations in 515 women with invasive cancers – 39 in *BRCA1*, 21 in *BRCA2*. The frequency of mutations among women with invasive cancers was 11.7 percent – higher than earlier estimates. Most hereditary ovarian cancers diagnosed in women under age 50 were due to *BRCA1* (83 percent); most of those diagnosed in woman over 60 were due to *BRCA2* (60 percent). The researchers found mutations in 19 percent of women who reported first-degree relatives with breast or ovarian cancer, and in 6.5 percent of women with no affected first-degree relatives. Risks of ovarian, breast, and stomach cancers and leukemias/lymphomas were increased 9-, 5-, 6-and 3-fold among first-degree relatives of women who had *BRCA1* mutations, compared with relatives of noncarriers. Risk of colorectal cancer was increased 3-fold for relatives of women who had *BRCA2* mutations. For carriers of *BRCA1* mutations, the estimated incidence of ovarian cancer was 36 percent by age 80.

Implications: Past studies may have underestimated the contribution of *BRCA2* to ovarian cancer because these mutations cause mainly late onset cancer, and earlier work targeted early-onset disease. The results of this larger study show that *BRCA2* mutations account for a significant subset of hereditary ovarian cancer cases.

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Racial and Ethnic Differences in Advanced-Stage Prostate Cancer

Background: The Prostate Cancer Outcomes Study of 3,143 men with prostate cancer answers important questions about the roles race, income, and other socioeconomic variables play in advanced prostate cancer. Data from NCI's Surveillance, Epidemiology and End Results (SEER) program – which gives information on U.S. cancer incidence and survival – show that, when they are diagnosed, African-Americans have twice the risk of non-Hispanic whites of being diagnosed with advanced prostate cancer (12.3 percent vs. 6.3 percent); Hispanics are intermediate (10.5 percent). Socioeconomic status and clinical and pathological factors each contributed 15 percent of the increased relative risk. After adjusting for all variables, the risk was still higher for African-Americans but not for Hispanics.

Advance: This research helps clarify health differences in advanced prostate cancer at diagnosis by race and ethnicity by focusing on the role of socioeconomic factors. Among men who had not finished high school or who had no private insurance, African-Americans and Hispanics were more likely to be diagnosed with advanced prostate cancer. However, socioeconomic status did not fully explain the increased risk seen for African-Americans; with higher socioeconomic status, the difference for Hispanics disappeared but persisted for African-Americans. Other variables associated with advanced prostate cancer in all men included high scores on a test that reports the tumor grade to determine how much of the tumor tissue differs from normal prostate tissue, public or no insurance, no documented prostate-specific antigen (PSA) test, and urinary
frequency.

Implications: The failure to explain tumor stage differences among racial and ethnic groups by looking at household income, employment status, education level, and insurance status suggests that either these widely used socioeconomic factors do not sufficiently describe the differences in access to and use of health care, or that biologic factors may have a role. Ultimately, efforts to reduce prostate cancer mortality in African-Americans should incorporate the racial difference in clinical stage at diagnosis. Further research should identify biologic markers, genetic susceptibility factors, and socioeconomic factors that include health care system use, distance from health care facilities, diet, literacy, and health beliefs.

Hoffman RM, Gilliland FD, Eley JW, Harlan LC, Stephenson RA, Stanford JL, Albertson PC, Hamilton AS, Hunt WC, and Potosky AL: Racial and ethnic differences in advanced-stage prostate cancer (APC): the Prostate Cancer Outcomes Study (PCOS). Journal of the National Cancer Institute 93: 388-395, 2001.

Genetic and Structural Studies of Apert Syndrome

Background: Apert syndrome is a genetic disorder characterized by premature closing of the skull's bony plates and severe webbing of the hands and feet. It occurs in approximately one in 160,000 to 200,000 live births. In common with many human skeletal disorders, Apert syndrome is caused by mutations in fibroblast growth factor receptors. Nearly all cases of Apert syndrome can be traced to two mutations in the fibroblast growth factor receptor 2 (FGFR2). Patients with a Ser252Trp mutation frequently have cleft palate, while those with a Pro253Arg mutation exhibit more severe webbing. Researchers think that both mutations alter the binding characteristics of FGFR2, leading to inappropriate activation of the receptor.

Advance: To understand the molecular mechanisms by which FGFR2 mutations cause Apert syndrome, NIH grantees prepared crystals of fibroblast growth factor 2 bound to the mutated receptors and to normal FGFR2, and defined their 3-D structures by X-ray crystallographic analysis. They found that both FGFR2 mutations resulted in stronger binding of the receptor to fibroblast growth factor 2. Increased binding affinity upsets the balance between receptor activation and inactivation, leading to inappropriate signaling. Based on the crystal structures, the researchers proposed that both mutations also increase the receptor's affinity for other fibroblast growth factors, including some it normally does not bind. The Pro253Arg mutation, they extrapolated, would allow FGFR2 to bind any of the 22 known members of the fibroblast growth factor family, while the Ser252Trp mutation would increase the receptor's ability to bind a limited subset of the growth factors. Based on the structural analyses, the researchers propose that changes in the binding affinity and specificity produced by the two FGFR2 mutations may account for differences in the physical anomalies seen in the two subgroups of Apert syndrome patients.

Implications: This report is believed to be the first to describe the crystal structures of mutant receptors implicated in human disease bound to their ligands. The study demonstrates the usefulness of structural biology for explaining the molecular basis of a genetic disorder. Besides providing a structural basis for understanding Apert syndrome and its phenotypic variability, these crystal structures also establish a framework for engineering artificial fibroblast growth factors for use in treating Apert syndrome as well as other growth regulatory disorders such as cancer.

Ibrahimi OA, Eliseenkova AV, Plotnikov AN, Yu K, Ornitz DM and Mohammadi M: Structural basis for fibroblast growth factor receptor 2 activation in Apert syndrome. Proceedings of the National Academy of Science USA 98: 7182-7187, 2001.

Human Brain's Natural Painkiller System in Action

Background: Research has supported the critical role of the mu opioid system, in which naturally produced chemicals called endogenous opioids, or endorphins, bind to receptors on brain cells to reduce or block the spread of pain messages from the body through the brain. The mu opioid receptor in particular has been found to be a major target for both the body's own painkillers, as well as for drugs such as heroin, morphine, methadone, and anesthetics, which also numb pain. NIH researchers used positron emission tomography, or PET, a technique that allowed them to have a unique window into the chemical activity of the volunteers' brains. Salt water was injected into the volunteers' jaw muscles, which caused jaw pain mimicking the chronic condition of temporomandibular joint disorder (TMD), and brain images were acquired. They confirmed the long-suspected connections between pain-dampening changes in brain chemistry and the senses and emotions experienced by people in pain.

Advance: This is the first study to analyze sustained pain with simultaneous brain scan monitoring of a key neurochemical system and the self-reported pain ratings of human participants. The study found that the onset and slow release of jaw muscle pain over 20 minutes caused a surge in the release of opioids. It also found that the flood of those chemicals coincided with a reduction in the amount of pain and pain-related emotions the volunteers said they felt. Specific brain regions, especially those already known to play a role in affective, or emotional, responses, and those known to help process signals from the body's sensory systems, had the biggest increase in the level of opioids when pain was introduced. The research also revealed major variation among volunteers in the baseline and pain-induced levels of opioids. The activation of the anti-pain response was dramatic in some volunteers when the placebo and pain-inducing conditions were compared, while in others the response was much less pronounced. Those who had the biggest change tended to rate the experience of pain the lowest, both in its sensory and emotional aspects.

Implications: This research provides new insights into the importance of the body's natural painkiller system and the reasons why each of us experiences pain differently. The results also show how brain chemistry regulates sensory and emotional experiences. The findings may help researchers better understand prolonged pain and find more effective ways to relieve it.

Zubieta JK, Smith YR, Bueller JA, Xu Y, Kilbourn MR, Jewett DM, Meyer CR, Koeppe RA, and Stohler CS: Regional mu opioid receptor regulation of sensory and affective dimensions of pain. Science 293: 311-315, 2001.

Interaction Between Genes having Tumor Suppressor and Oncogenic Functions

Background: The p53 gene and its products are recognized as regulators of cell growth and differentiation and function in programmed cell death, or apoptosis, thus also serving as tumor suppressors. It is the most commonly inactivated gene in cancers, including those of the head and neck. In human cancers, mutant p53 gene products are unable to bind specific DNA sequences and activate responsive genes that arrest cell growth or induce apoptosis. This loss of critical regulatory and signaling functions is central to tumor formation, growth and metastasis.

During embryonic development, p53 is down-regulated so that the essential cellular growth and differentiation functions of other genes can proceed. One such gene, p63, plays an essential role during embryonic development of craniofacial and other epidermal tissues. Mutation of this gene in human embryos causes the defective development of skin and craniofacial structures, including teeth, known as ectodermal dysplasia. In squamous cell carcinomas, primary lung cancers, and in head and neck small cell cancer cell lines, the p63 gene is frequently amplified and certain of its isotypes, including □Np63, are overexpressed and display cancer-causing (oncogenic) properties.

Advance: Based on the similarity of DNA binding and other molecular structures within the p53 gene family and its homologues, NIH grantees examined the physical and functional relationships between p53 and the p63 isotype, □Np63. Their findings clearly demonstrate that □Np63 forms complexes with p53, both in vitro and in vivo, providing evidence that p53 is a true protein-binding partner. In addition the researchers found that p53 stimulates the degradation of □Np63, and does so by activation of the enzyme, caspase 1.

Implications: These findings support the concept that members of the p53 gene family and their homologues interact to regulate their biological functions. The data suggest that p63 functions as a negative regulator of some p53 genes, while p53 plays an important role in the control of p63. Consequently, the regulation of cell growth during embryonic development and cancer development most likely depends on a precise balance between tumor suppressive and tumor promoting members of the p53 family. Further definition of this balance may provide a means to perturb or modify it to control tumor development.

Ratovitski EA, Patturajan M, Hibi K, Trink B, Yamaguchi K,and Sidransky D: p53 associates with and targets □Np63 into a protein degradation pathway. Proceedings of the National Academy of Sciences USA 98: 1817-1822, 2001.

Mouse Model Sheds Light on Hereditary Dental Defect

Background: Amelogenesis imperfecta (AI) is a hereditary disorder characterized by abnormal development of enamel, the hard outer covering of the tooth, resulting in enamel that is defective in structure or deficient in quantity. Dental enamel, composed of a highly organized calcium/phosphate crystal, is the hardest substance in the body and cannot be replaced since the cells that secrete it do not exist after tooth eruption. During the development of the enamel, the amelogenin proteins make up about 90 percent of the extracellular matrix. Scientists believe amelogenins have an important role in enamel formation, although their precise functions are not well understood, and that a defective amelogenin gene is the cause of AI.

Advance: To better understand the functions of amelogenin proteins, NIH researchers have created a mouse model that mimics amelogenesis imperfecta. The researchers produced the mice by deleting, or knocking out, the amelogenin gene. The knockout mice displayed abnormal teeth with chalky white discoloration as early as two weeks of age and an abnormally thin layer of tooth enamel. Through scanning electron microscopic analysis, the researchers also found that the enamel lacked the "prism" pattern that is the hallmark of normal enamel crystal. These findings revealed that the amelogenins are apparently not required for initiating enamel formation, but rather are necessary for the organization of the distinctive crystal pattern and the regulation of enamel thickness.

Implications: This mouse model will be useful for understanding the functions of amelogenin proteins during enamel formation and for developing therapeutic approaches for treating amelogenesis imperfecta.

Gibson CW, Yuan ZY, Hall B, Longenecker G, Chen E, Thyagarajan T, Sreenath T, Wright T J, Decker S, Piddington R, Harrison G, and Kulkarni AB: Amelogenin deficient mice dispaly an amelogenesis imperfecta phenotype. Journal of Biological Chemistry 276(34): 31871-31875, 2001.

Natural Genetic Transformation of Oral Bacteria

Background: Natural genetic transformation is a process by which bacteria incorporate the DNA freed from other bacteria in their environment and thus acquire new genes that enable them to adapt to changing environments and potentially develop antibiotic resistance, virulence and other genetically controlled traits. Recent evidence suggests that gene transfer between bacterial species can be facilitated by their growth in oral plaques or biofilms.

Advance: NIH-supported scientists have studied *Streptococcus mutans* (*S. Mutans*), a bacterial species that naturally grows in dental biofilms, to identify the biochemical pathways and the enabling genetic mechanisms involved in natural genetic transformation by this organism. Using genomic analyses, they demonstrated that *S. mutans* uses a peptide signaling pathway similar to that of other streptococci to stimulate the uptake and incorporation of foreign DNA. Their results suggest that the oral biofilm or dental plaque may provide optimum conditions for the signaling system and, consequently, for genetic transformation.

Implications: The concept that dental plaque may provide streptococci with a vast reservoir of genetic information has a serious implication in that the potential exists for the transfer of antibiotic resistance to pathogens that may transiently reside in dental biofilms.

Li YH, Lau PCY, Lee JH, Ellen RP and Cvitkovitch D: Natural genetic transformation of *Streptococcus mutans* growing in biofilms. Journal of Bacteriology 183: 897-908, 2001.

Scientists Sequence Genome of Major Periodontal Disease Bacterium

Background: By conservative estimate, more than 35 million Americans have periodontitis, a chronic infectious disease of the gums and underlying bony tissues. Untreated, it can destroy those tissues and result in tooth loss. A small number of gram-negative bacteria are associated with specific forms of periodontitis. *Porphyromonas gingivalis* (*P. gingivalis*) is the organism associated with chronic and severe adult periodontitis.

Advance: NIH-supported scientists have now sequenced the genome of *P. gingivalis.* It is the first oral disease-causing microbe to be completely sequenced. The annotated *P. gingivalis* sequence has been posted on the Internet, making it freely available to researchers worldwide. The sequence provides the scientific community at large with information on an organism from a major group of bacteria not previously sequenced.

Implications: With the genetic blueprint for *P. gingivalis* in hand, dental researchers will be able to identify potential targets for periodontal vaccines and drug therapies.

www.pgingivalis.org

Bringing Sleep into Focus

Background: Recent research on the developing visual system in animals has provided direct evidence that sleep in early life plays a crucial role in brain development. In normal animals the numbers of cortical neurons dominated by inputs from each eye are roughly equivalent. Neurons receiving input from the same eye are grouped in aggregates in the visual cortex called ocular dominance columns. Innovative research previously showed that during a critical period of development, closing one eye (monocular deprivation or MD) dramatically shifts the balance of ocular dominance so that only a few neurons receiving input from the deprived eye remain. When the critical period has ended, reversing this imbalance is very difficult. Recent work using this system has shown that significant shifts in ocular dominance can occur with periods of visual deprivation lasting as short as a few hours.

Advance: Visual deprivation was used to develop an assay for the effects of sleep on neural plasticity in cats. In a control group, experimental measurements of ocular dominance in the cortical surface were done after a standard MD treatment. Experimental groups were given one of three different treatments for a six-hour period following MD. One group was allowed to sleep at will in total darkness, a second group was kept awake in total darkness, and a third group was kept awake in the light. The control animals showed the expected shift in optical dominance toward the open eye. Sleep enhanced the effects of MD on visual cortical responses, but wakefulness, even in complete darkness, did not do so.

Implications: Despite all the research on sleep, we know relatively little about its effects on the brain. We know that even minor sleep deprivation can have a devastating effect on mental performance, and a host of other essential functions. One of the proposed functions of sleep is memory consolidation. This theory proposes that sleep is a period of low sensory input during which the brain consolidates events of recently acquired tasks. The present study shows that during development, sleep allows the consolidation of changes in ocular dominance evoked by short-term visual experience. Sleep deprivation prevents consolidation of the visual experience and appears to allow accumulated changes to reverse. The results provide the first direct evidence that sleep and sleep deprivation modify experience-dependent changes in the brain, and also suggest that synaptic circuits are modified during sleep. This study may be an opportunity for future explorations of the mechanisms underlying sleep and hopefully a clearer understanding of the function of sleep.

Frank MG, Issa NP, and Stryker MP: Sleep enhances plasticity in the developing cortex. Neuron 30: 275-287, 2001.

Where does Visual Plasticity Occur in the Brain?

Background: Ocular dominance columns are columns of nerve cells in the visual cortex that respond to visual input and activity from one eye or the other in binocular animals. In studies of visual system plasticity (its ability to be molded by visual input) changes in the ocular dominance columns in the visual cortex are a hallmark indicator of plastic changes during development. The long standing belief has been that ocular dominance columns emerge *de novo* during development from an initial state where visual inputs from the part of the brain known as the lateral geniculate nucleus (LGN), representing the two eyes, change from an overlapping representation to separate columnar aggregates each representing the input from one eye. This process is believed to take place during a limited period in development called the critical period. Experimental observations of this organization were made using injections of nerve tracers that were restricted to single layers of the LGN, with each layer of the LGN receiving its input from one eye. However, interpreting these observations are complicated by the fact that the presence of a continuous band of label in the young cortex could either be due to the absence of segregated ocular dominance columns, or due to spillover to more than one layer of the LGN.

Advance: In a recent study, scientists showed that ocular dominance columns in the visual cortex of the ferret appear long before the columns can be modified by visual experience during brain development. The use of the ferret as an experimental model is critical for this new observation. The visual system of the ferret is at a much earlier stage of development at birth than the cat, commonly used for these studies. Unlike the cat, the projection of the nerve processes from the LGN, in the thalamus, to the visual cortex develop after birth in the ferret, allowing studies that would otherwise be difficult. Tracer injections confined to individual LGN layers produced clearly segregated patches of labeled cells in the developing cortex as early as postnatal days 16 to 18 in the ferret. Projections from restricted injections to both layers of the LGN gave rise to separate labeled patches in the cortex at this early stage of development in the ferret. The labeled cortical patches have all the characteristics of ocular dominance columns observed in the adult. To further test the effects of imbalanced inputs of visual activity on ocular dominance columns, monocular enucleations were done on young animals. In all cases, the patchy cortical labeling arising from injections in the LGN persisted.

Implication: These observations show that ocular dominance columns appear much earlier than previously thought, and at a much earlier stage of visual cortical development. Earlier studies on the monkey suggest a similar finding. Newborn monkeys have ocular dominance columns very similar to those in the adult. Ocular dominance columns appear to be established before they can be modified by visual experience, or put another way, the plastic changes associated with visual experience during the critical period act on pre-existing cortical columns. This new research suggests that neural activity is not required for the establishment of cortical columns, instead molecular cues guide their formation, although neural activity clearly modifies them later during the critical period. These results may also suggest that the establishment and plasticity associated with ocular dominance columns are at different stages of visual system development.

Crowley JC, and Katz LC: Early development of ocular dominance columns. Science 290: 1321-1324, 2001.

The Timing of Visual Responses to Light

Background: The ability to perceive changes in a visual scene requires that the visual system be able to detect or resolve the changes in both space and time. Therefore, one of the main functions of the retina is to separate visual information into these spatial and temporal components. Retinal photoreceptor cells, which capture light, are physically separated within the retina, and this separation accounts for considerable spatial resolution. However, photoreceptor light responses are uniform in their time course; and only a single neurotransmitter substance, glutamate, appears to be released by photoreceptors. Thus, temporal resolution must occur somewhere else within the millions of neurons in the retina and brain that comprise the visual system. Several different types of specialized neurons called bipolar cells are known to receive neural impulses from the photoreceptors. But it is not known why there are so many distinct types when only a single neurotransmitter chemical is released onto them.

Advance: Measurements of electrical activity inside the cell made from single, distinct types of bipolar cell demonstrated that each distinct type of neuron uses a different and distinct receptor subtype to bind the glutamate released by the photoreceptors. Each glutamate receptor subtype responds with a different and unique time course, shaping the bipolar cell's subsequent response to glutamate released by the photoreceptors. Thus, diverse glutamate receptor subtypes with different functional properties begin the process of temporal resolution at the visual system's first synapse, the point at which neural impulses are passed between neurons.

Implications: The photoreceptor/bipolar cell synapse is the first site in the retina at which visual signals can flow into parallel processing pathways, and detailed behavior at this synapse is governed by turnover in receptor kinetics. Whether these different glutamate receptor subtypes are associated with different morphological types of synapses at photoreceptors is an interesting question yet to be resolved.

DeVries SH: Bipolar cells use kainate and AMPA receptors to filter visual information into separate channels. Neuron 28: 847-856, 2000.

Early Eye Development

Background: Development of the vertebrate eye is controlled by specific genes that operate in a hierarchy of expression. A number of these genes have been identified as "master controls". In Drosophila, the fruit fly, loss of any one master control gene results in the failure to form an eye, while the misexpression of any one is sufficient to form an eye in aberrant body locations. One of these Drosophila master genes, eyeless, is similar to a human gene, Pax-6. Pax-6 mutations result in aniridia, a congenital malformation of the eye associated with improper development of the iris and with the formation of cataracts. Pax-6/eyeless genes are found in other embryonic tissues, and they are crucial to the formation of other organ systems, such as the nose/antenna. A key issue in understanding the function of master genes is to delineate the factors, which turn on each tissue-specific developmental program.

Advance: Recently, two signaling pathway receptors were identified in Drosophila that act before eyeless to specify eye formation. One is the transmembrane receptor, Notch that promotes eye formation. The second is the EGF receptor that blocks eye formation in favor of antennae. These findings are the first to suggest a mechanism of global control of eye development.

Implications: Identifying developmental genes and their products is essential to the understanding of the developmental hierarchy controlling ocular development and will enhance our understanding of the molecular basis of congenital diseases of the eye.

Kumar J, and Moses K: EGF receptor and notch signaling act upstream of eyeless/Pax-6 to control eye specification. Cell 104: 687-697, 2001.

Discovery of Gene for Hallervorden-Spatz Syndrome

Background: Hallervorden-Spatz syndrome (HSS) is a rare, inherited, neurological disorder associated with high accumulations of iron in the brain, and causes progressive degeneration of the retina and nervous system. Symptoms of HSS can vary widely and include involuntary, jerky muscle movements; uncontrolled tightness of the muscles; and sudden, involuntary muscle spasms (spasticity). A number of patients develop degeneration of the retina. The symptoms usually develop during childhood, most often between the ages of two and 15 years. Death usually occurs in early adulthood, approximately 10 years after onset. Currently, there is no effective treatment.

Advance: Scientists recently discovered a defective gene, PANK2, that produces an ineffective enzyme in patients with HSS. The normal gene produces an enzyme known as pantothenate kinase. The enzyme is needed by the body to use vitamin B5, which is required to produce some of the body's essential compounds. These researchers hypothesize that the production of the ineffective enzyme by the defective gene causes blockage of normal metabolism and the accumulation of metabolic materials resulting from that blockage. It is believed that this accumulation results in degeneration of the retina and a high concentration of iron in the neural tissues.

Implications: Research can now be focused on developing treatment strategies that bypass this defective enzyme, allowing the body to use vitamin B5 to help make essential components. Understanding the biochemical defects in HSS may also provide insights into the effect iron has on other neurodegenerative diseases associated with high iron accumulations, such as Parkinson's disease.

Zhou B, Westaway SK, Levinson B, Johnson MA, Gitschier J, and Hayflick SJ: A novel pantothenate kinase gene (PANK2) is defective in Hallervorden-Spatz syndrome. Nature Genetics 28: 345-349, 2001.

Protection of the Lens by αα-Crystallin

Background: Lens transparency requires high concentrations of protein within specialized lens fiber cells. Because fiber cell proteins are not removed or replaced during the life of an individual, the lens is especially susceptible to aging effects. As these proteins age, changes occur that cause them to aggregate into large, opaque structures that can interfere with vision. To maintain transparency, the lens must maintain the integrity of its proteins. In general, cells use a chaperone protein to protect other proteins when the cell is subjected to stress. In the lens, α-crystallin, a protein belonging to a family of chaperones, is composed of two subunits αA- and αB-crystallin that also function as chaperones. Although αA-crystallin is primarily found in the lens, αB-crystallin is broadly distributed in other tissues. Studies have shown that they both have a number of other physiological functions in non-ocular tissues beside their important ocular role of preventing protein aggregation during aging. Early in development, αA- and αB-crystallin are found in lens epithelial cells at low levels, but there is a substantial increase in αα-crystallin during differentiation into fiber cells. Studies of lens epithelial cells grown in the laboratory from animal models lacking αA- and αB-crystallins suggest that they also play a role in controlling cellular growth.

Advance: Further investigation of the protective roles of αA- and αB-crystallin in epithelial cells led scientists to test the capability of αA- and αB-crystallin to protect human epithelial cells from apoptosis (programmed cell death). They found that introduction of αA-crystallin encoding DNA in these cells protected them from a variety substances that are known to cause apoptotic cell death. They also investigated the effect of introducing αA-crystallin and αB-crystallin DNA into lens epithelial cells grown in the laboratory from animals without these genetic sequences. Differing levels of expression of the DNA led to isolation of cells with differing amounts of αA- and αB-crystallin. By subjecting these cells to conditions of apoptotic stress, they learned that αA-crystallin is two to three times more effective than αB-crystallin in protecting lens epithelial cells from stress-induced cell death.

*Implication*s: Both αA- and αB-crystallins are major components of the fiber cells of vertebrate lenses and are found in lower levels in the metabolically active lens epithelial cells. The results of this work suggest that an important role of αA-crystallin in the lens, the tissue in which it is primarily expressed, is to protect the lens from stress-induced cell death. Understanding the range of protective mechanisms available to the lens may assist in determining the events that overwhelm them and lead to formation of cataracts.

Andley UP, Song Z, Wawrousek EF, Fleming TP, and Bassnett S: Differential protective activity of □A- and □B-crystallin in lens epithelial cells. Journal of Biological Chemistry 275: 36823-36831, 2001.

Myocilin in Aqueous Humor

Background: Glaucoma is a potentially blinding eye disease that affects approximately three million Americans, with as many as 120,000 now blind from this disease. The disease is most commonly associated with elevated intraocular pressure; however, the precise cause of this increase in pressure in the eye is not known. Considerable evidence points to a blockage at the site from which fluid, known as aqueous humor, flows out of the eye as the cause of pressure elevation. The tissue that regulates the exit of fluid from the eye, the trabecular meshwork, is composed of endothelial cells (specialized cells that line blood vessels and other tissues) and noncellular components. Recently, scientists have located a mutation in a gene on chromosome 1 that is linked to the most common form of this disease, primary open angle glaucoma. This gene directs production of myocilin, a protein found in the trabecular meshwork. It is also found in other eye tissues.

Advance: Researchers wanted to know whether myocilin was also present in the aqueous humor, the fluid responsible for the build up of pressure within the eye. They analyzed the aqueous humor from human, monkey, and cow eyes and found that myocilin was a component of the aqueous humor in each. They found that the protein was larger than expected and was hydrophobic or unable to mix with water. The scientists also found that the proteins became tightly adherent to filters that become obstructed when aqueous humor was passed through them.

Implications: Myocilin is a component of aqueous humor, and its relatively large size may indicate that it contains repeating units or is in association with other proteins that are present in the aqueous humor. The molecule's large size and the possibility that it may be involved in the obstruction of filters of the size of the tissues involved in aqueous humor outflow from the eye are intriguing and warrant additional study.

Russell P, Tamm ER, Grehn FJ, Picht G, and Johnson M: The presence and properties of myocilin in the aqueous humor. Investigative Ophthalmology and Visual Science 42: 983-986, 2001.

Growth Factors Prevent Alcohol's Brain Damage in Living Mammal Fetus

Background: Alcohol is, by far, the greatest inducer of birth defects, compared with any of the illegal drugs in use today. It often manifests as fetal alcohol syndrome (FAS) or fetal alcohol effects (FAE) in children of women who drink during pregnancy. Both of these conditions may result in life-long, debilitating neurologic damage and behavioral deficits.

We are identifying mechanisms through which alcohol damages the fetal nervous system. One of these mechanisms is cell death ("apoptosis"). Some fetal brain cells are programmed to die as a normal part of development. However, the kinds of cells that die and the timing of their demise are critical. When these factors are thrown off – by alcohol molecules interfering with biochemical reactions that occur in the fetal brain, for example – birth defects can result.

Among the substances that regulate apoptosis are neurotrophic factors. Studies have shown that alcohol compromises neurotrophic factors, particularly during certain stages of fetal development. In studies of nerve cells exposed to alcohol in test tubes and in chicken embryos, addition of one of these neurotrophic factors, nerve-growth factor, enhanced cell survival.

Scientists asked if increasing fetal production of nerve-growth factor would attenuate alcohol-induced damage of fetal brain cells, not in test-tubes or chicken embryos, but in living mammals (mice). They genetically engineered mice to over-produce nerve-growth factor as the mice developed. Next, they exposed the mice to alcohol at a time when cells of the cerebellum, the part of the brain that coordinates movement, are most sensitive to alcohol's toxic effects.

Advance: For the first time in a living mammal model, scientists showed that increased production of nerve-growth factor protected a sensitive brain region against excessive cell death during exposure to alcohol. This held true even during a developmental stage when the cells usually would have been particularly vulnerable to alcohol-induced damage.

Implications: We now have evidence that neurotrophic factors protect developing nerve cells from the damaging effects of alcohol not only in culture dishes, but also in living mammals. If we find that enhanced production of other neurotrophic factors prevents alcohol-induced brain damage or that neurotrophic factors protect other alcohol-sensitive brain regions, we may be able to develop therapeutic *in-utero* treatments based on this research.

Heaton MB, Mitchell JJ, and Paiva M: Overexpression of NGF ameliorates ethanol neurotoxicity in the developing cerebellum. Journal of Neurobiology 45: 95-104, 2000.

An Appetite for Alcohol

Background: Very often, different research disciplines are interested in studying the same issues, but for different reasons. Their findings build on each other. The findings described here illustrate just such a case, in which investigators from one NIH Institute built on research funded by investigators from another NIH Institute.

A number of disciplines are interested in biologic mechanisms that regulate appetite, mostly because of the health consequences of obesity; for example, diabetes and heart disease. Alcohol researchers are interested in these mechanisms for another reason. Some of the same mechanisms that regulate appetite for food also appear to be involved in regulating appetite for alcohol and appear to play a role in risk of alcoholism.

Appetite is much more than the brain simply telling the body to eat when the stomach is empty. It's a complex orchestration, in which the hypothalamus of the brain gets biochemical messages about the body's nutrition and energy state, then responds by adjusting appetite and balancing the body's use of energy. Leptin, a hormone made by fat cells, is the chief signaler of information to the hypothalamus about the body's nutrition and energy status. It modulates appetite by reducing the activity of genes that produce appetite-promoting substances.

Marijuana increases appetite. Researchers asked whether endocannabinoids, the brain's own natural marijuana-like substances, have a similar appetite-promoting effect and whether leptin regulates them. These substances, anandamide and 2-arachidonolyl glycerol, are present in the hypothalamus, the brain's appetite-regulating center. So are cellular receptors that bind with the endocannabinoids, triggering their actions; they are called "CB1 cannabinoid receptors."

Investigators eliminated the CB1 receptor, in mice, by eliminating the gene that carries the blueprint for it. They compared them with unaltered mice. They also treated unaltered mice with a chemical compound (SR141716A) that blocks the receptor, making it ineffective. In each of the experiments, the investigators briefly withheld food from the mice, to induce hunger.

Advance: Animals missing the CB1 receptor ate less than did their unaltered littermates. Unaltered mice ate less if they had been given SR141716A. This finding was built on by testing leptin-deficient, obese mice Researchers found that they had high levels of endocannabinoids in the hypothalamus and that these levels dropped when we gave them leptin (which also occurred when we gave leptin to normal rats).

Implications: Endocannabinoids in the hypothalamus appear to be part of the leptin-regulated neural circuits – networks of nerve cells and the thousands of biochemical reactions associated with them – that govern appetite. The findings described here suggest that endocannabinoids promote eating by activating the CB1 receptor. This research is of value to disciplines with an interest in altering pathologic patterns of appetite, whether for food, alcohol, or other substances.

DiMarzo V, Goparaju SK, Wang L, Liu J, Bátkai S, Járai Z, Fezza F, Miura GI, Palmiter RD, Sugiura T, and Kunos G: Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature (in press 2001).

Craving for Alcohol Activates Specific Brain Areas in Alcoholics

Background: To understand how best to treat alcoholism, we must understand the physical phenomena that underlie the disease. Craving for alcohol is a hallmark of the disease, and we are exploring craving-related changes in the brain's structure and function.

A difficulty in gathering these kinds of data, however, is that people's self-reports of craving for alcohol tend to be less than that for other substances. If scientists could observe changes that accompany craving in specific areas of the brain, this would provide a valuable research tool. More important, it would point to structural and functional areas of the brain involved in a key component of alcoholism.

NIH-funded researchers compared functional magnetic resonance imaging (fMRI) studies of the brains of alcoholics and social drinkers. During the test, investigators gave members of each group a sip of alcoholic beverage, then showed them pictures of alcoholic and nonalcoholic drinks as cues to elicit craving.

Advance: In response to the craving cues, alcoholics' brains had increased activity in specific brain regions: the left dorsolateral prefrontal cortex and the anterior thalamus. The brains of social drinkers did not have these changes.

Implications: We are in the early stages of identifying brain areas involved in the complex behaviors associated with alcohol use. The regions that became active in the alcoholic subjects' brains in response to craving cues regulate emotions, attention, and appetite. We know that all of these factors probably are involved in alcohol-drinking behaviors. The brain areas the investigators identified may or may not be the *cause* of craving for alcohol, but we now know that they are somehow associated with it.

Other studies have identified brain areas that become active in response to craving for certain illegal drugs. Some of them are the same as the areas that became active in response to alcohol-craving, but alcohol-craving appears to stimulate other, different areas, as well.

George MS, Anton RF, Bloomer C, Teneback KC, Drobes DJ, Lorberbaum JP, Nahas Z, and Vincent DJ: Activation of prefrontal cortex and anterior thalamus in alcohol subjects on exposure to alcohol-specific cues. Archives of General Psychiatry 58: 345-352, 2001.

Alcoholics Have Changes in Activity of Brain's "White-Matter" Genes

Background: The brain adapts to chronic alcohol use by building up a physiologic tolerance to it, which progresses to a physiologic dependence. A second scenario ensues: as people drink larger amounts to try to achieve the same rewarding sensations, their brain tissues are exposed to greater amounts of alcohol. Unlike other drugs of abuse, alcohol is a toxin that can damage any tissue in the body, including brain tissue. With increasing amounts of alcohol, people are more exposed not only to its addicting properties, but also to its toxic, tissue-damaging properties. Some parts of the brain may shrink, with debilitating changes in memory and cognition.

In the frontal lobe of the brain, shrinkage results from alcohol-induced loss of white matter. This white matter, myelin, is a kind of insulation that wraps around certain nerve cells, to enable them to transmit electrical "messages" at the speed required. Since myelin is made not only of fat, but also of protein, genes enter the picture here. Genes hold the blueprints for production of proteins, including myelin proteins. When a gene is activated, it triggers cellular mechanisms that cause the cell to make the protein the gene "told" it to make. The activation of the gene and subsequent production of the protein are called "gene expression." In the study described here, expression of myelin-producing genes played a prominent role.

Investigators took autopsy samples from the frontal cortices, part of the frontal lobe of the brain, from alcoholics and nonalcoholics. They tested them with a recent genetics technique, microarray technology, that enables scientists to detect changes in expression of thousands of genes at once. They tested the expression of 4,000 different genes.

Advance: Gene expression in alcoholics and nonalcoholics differed by 40 percent in 163 of the genes studied. The alcoholics' tissue samples were especially notable for their decreased expression of genes holding the blueprints for myelin.

Implications: We do not know whether alcohol affects myelin-producing genes themselves or disrupts other factors that contribute to the normal function of these genes. We do know that the frontal cortex, the frontal-lobe site from which researchers took the autopsy samples, plays an important role in decision-making and judgment – functions that often are deficient in alcoholics. A potential explanation for these deficits could be that alcohol-induced changes in gene expression cause changes in neural circuits – networks of nerve cells – of the frontal cortex.

Lewohl JM, Wang L, Miles MF, Zhang L, Dodd PR, and Harris RA: Gene expression in human alcoholism: microarray analysis of frontal cortex. Alcoholism, Clinical and Experimental Research 24: 1873-1882, 2000.

Closing in on Genes for Alcoholism

Background: Between 50 and 60 percent of the risk of alcoholism is genetic. We know this from studies of alcoholic families. We also know that each gene is a blueprint for the production of specific proteins and that these proteins take part in thousands of biochemical reactions that determine physical traits and behaviors, including behaviors related to alcohol use. When a gene varies from "normal," so does the protein it produces ("encodes"). That can throw off the biochemical reactions in which that protein takes part, thus changing physical traits or behaviors; for example, a variant protein can result in higher tolerance of alcohol, which can result in heavy drinking. Chronic, heavy drinking leads to adaptations in the brain that form the physical basis of alcohol dependence.

What we do not know is exactly which genes are involved in alcohol-related behavior. We do, however, have suggestive evidence of the general locations, on certain chromosomes, of some of them. In the past, the NIH's Collaborative Study on the Genetics of Alcoholism (COGA) conducted genome scans on a large group of families with alcoholic members. Results suggested that some of the risk of alcoholism lies in genes on chromosomes 1, 2, and 7.

Advance: COGA investigators added to the evidence that some of the genes involved in alcoholism are on chromosomes 1 and 7 by repeating their findings in a genome scan of a second, large group of alcoholic families.

Implications: These kinds of results are not definitive until they have been replicated in a number of reliable studies. With this finding, evidence implicating chromosomes 1 and 7 in alcoholism is mounting.

Of interest to researchers is that both chromosomes 1 and 7 contain genes that other kinds of studies have identified as encoding proteins that might mediate alcohol's actions in some way. For example, some of these proteins act as gatekeepers for cells, allowing only certain substances in and out, to regulate the cells' ability to conduct electrical and chemical messages. Alcohol is known to affect how these proteins function; in some cases, it inhibits these messages, resulting in the sedation that any of us who have felt sluggish after drinking alcohol would recognize. A gene on chromosome 7 is known to encode a piece of protein, neuropeptide Y, that affects appetite for alcohol in studies of mice.

Foroud T, Edenberg HJ, Goate A, Rice J, Flury L, Koller DL, Bierut LJ, Conneally PM, Nurnberger JI, Bucholz KK, Li TK, Hesselbrock V, Crowe R, Schuckit M, Porjesz B, Begleiter H, and Reich TR: Alcoholism susceptibility loci: confirmation studies in a replicate sample and further mapping. Alcoholism, Clinical and Experimental Research 24: 933-945, 2000.

New Non-invasive Method to Diagnose Pneumocystis carinii in Patients with HIV and Other Immunosuppressive Disorders

Background: Pneumocystis carinii pneumonia is the most common AIDS defining opportunistic infection, as well as a complication of chemotherapy for cancer and of organ transplantation. This pneumonia is fatal if untreated. Even with treatment, patients can die if appropriate therapy is not instituted promptly. The diagnosis must be established at most institutions by bronchoscopy, an invasive and uncomfortable procedure that health care professionals are often reluctant to recommend unless they have a very high suspicion of the disease. A less invasive means of diagnosis would be advantageous. Sputum analysis is used at some institutions, but many institutions either do not have sputum induction available, or they have poor success in recognizing disease.

Advance: Scientists have discovered that if an ultrasensitive nucleic acid detections system is used, pneumocystis can be detected in oral gargles, and bronchoscopy or sputum induction may not be needed. Prior investigators have also used nucleic acid systems in oral gargles, but the current technique uses a multicopy gene (a gene with more than one copy) as a target: this gene was discovered at NIH. The multicopy gene-based system is more sensitive than other techniques, and the detection system developed does not use radioactive probes, a big advantage for commercial laboratories because there are fewer safety, disposal, and expense issues involved.

Implications: Pneumocystis pneumonia can be readily detected using a non-invasive diagnostic technique, and this method can be used widely by laboratories that do not necessarily have specialized expertise recognizing this organism. This will allow earlier and more accurate diagnosis of this pneumonia, thus improving survival for patients with AIDS and other immunosuppressive disorders.

Fischer S, Gill VJ, Kovacs J, Miele P, Keary J, Silcott V, Lucey D, and Masur H: The use of oral washes to diagnose Pneumocystis carinii pneumonia: a blinded prospective study using a pcr based detection system. Journal of Infectious Diseases (in press 2001).

Resistance Testing to Optimize Antiretroviral Therapy in HIV-Infected Patients Who Have Failed Prior Therapy

Background: Highly active antiretroviral therapy (HAART) has improved the quality and duration of survival for patients with HIV infection, yet at least 50 percent of treated patients will fail therapy within 2 years, because their virus will become multidrug-resistant. Although there are 14 licensed drugs for treating HIV, it is often difficult to choose an effective regimen for patients who have failed several different drug combinations. Tests are now available to measure viral resistance, but it has not been clear how useful these expensive ($400 each) tests are.

Advance: In a study of 101 drug-experienced patients, both the genotype assay and the phenotype assay were compared with virologic response (appearance or disappearance of the virus in the blood). The more mutations to the nucleoside class of drugs that were detected by the genotype assay, the less likely the patient was to respond to a regimen of abacavir, amprenavir, and efavirenz, especially if the patient had been treated with efavirenz or related drugs in the past. Phenotypic resistance was also helpful.

Implications: Both genotypic and phenotypic resistance assays can be useful in some drug-experienced patients and thus should be performed. However, in many patients who have been extensively treated in the past with many drugs, such assays are unlikely to reveal a drug regimen that will be helpful.

Falloon J, Ait-Khaled M, Thomas DA, Brosgart CL, Eron JJJr, Feinberg J, Flanigan TP, Hammer SM, Kraus PW, Murphy R, Torres R, Masur H, and the CNA 2007 Study Team: HIV-1 genotype and phenotype correlate with virologic response to abacavir, amprenavir, and efavirenz in treatment experienced patients. AIDS (in press 2001).

Nitric Oxide Treatment Replenishes Blocked Nitric Oxide Synthesis and Maintains Vascular Blood Flow

Background: Nitric oxide is a naturally occurring compound, produced in the lining of blood vessels. It is known to be important in the regulation of coronary and systemic vascular tone. In experimental situations where synthesis of nitric oxide is blocked, decreases in coronary and systemic blood flow have been noted. Recently, NIH scientists demonstrated that hemoglobin has a role in nitric oxide transport, and that it may be linked to oxygen binding, promoting delivery of both oxygen and nitric oxide to regions in the body with low levels of oxygen. With nitric oxide inhalation, the compound seems to be stabilized and is able to be delivered to distant sites in the body where it may have a physiologic and therapeutic effect. Investigators recently studied whether inhalation of nitric oxide facilitates its transfer in the blood and whether it has an effect on vascular blood flow in areas distant from the lungs and in the setting of inhibition of its normal synthesis.

Advance: Scientists discovered that when normal synthesis of nitric oxide by the lining of blood vessels is inhibited and nitric oxide is given by the inhalation method, vascular blood flow can be restored to near normal levels. In this setting, NIH investigators also demonstrated significant metabolic changes, consistent with improved blood flow and tissue perfusion. Data also further strengthened the concept that inhaled nitric oxide gas can be stabilized after entering the lungs and thus be transported in blood and improve peripheral vascular blood flow.

Implications: Other investigators have demonstrated that in certain disease states, such as atherosclerosis, nitric oxide may be present in low levels, and this may explain some of the physiologic and clinical abnormalities that are noted. By providing a new source of nitric oxide, namely by inhalation of the gas, and getting it transported in the vasculature, blood flow may be restored. This effect may have clinical applications in the treatment of diseases characterized by decreased nitric oxide production, such as atherosclerosis and coronary artery disease.

Cannon RO, Schechter AN, Panza JA, Ognibene FP, Pease-Fye ME, Waclawiw MA, Shelhamer JH, and Gladwin MT: Effects of inhaled nitric oxide on regional blood flow are consistent with intravascular nitric oxide delivery. Journal of Clinical Investigation 108: 279-287, 2001.

Mosquito Larva, *Anopheles arabiensis*, Growth in the Presence of Maize Fields

Background: reater than half of all deaths from malaria in the world, now estimated to approach 3 million per year, occur among the lowest quintile of the poorest of the world's population. The proportion of deaths due to malaria in this population is greater than that for any other disease. As a vector borne infection, it is critical to understand the behavior of the responsible transmitting anopheline mosquitos. For example, their feeding patterns vary widely by anopheline species and geographic location. While some anophelines develop in bacteria-rich, clear water surface environments, the *Anopheles gambiae* family of African mosquitoes breed in turbid waters, which are generally vegetation free. The feeding strategies of *A. gambiae* has remained generally unexplored. One important question has been whether or not pollen plays a crucial role in the development of the mosquito larvae. Tropical plants in sub-Saharan Africa are generally pollinated by insects rather than wind so that pollen is relatively scarce in this area of the world. Maize, however, is a common crop in Africa that survives in a wide range of climates and altitudes and its pollen can be distributed by the wind. An increased understanding of the development of mosquito larvae as related to maize pollen is important in the development of malaria control strategies.

Advance: In a preliminary series of observations, NIH-supported investigators from Harvard University and counterparts in Ethiopia recorded the type of food ingested by the larval stage of the mosquito that carries malaria, the distribution of maize pollen in the vicinity, and the physical appearance of the water where mosquitoes breed. Maize pollen was observed on both the ground and water surface of breeding sites and was isolated from the gut of mosquitoes, suggesting that maize pollen is a food source. Researchers also compared the development of the larvae in water located 100 meters from maize plantings to sites directly adjacent to maize plantings. Evidence of larvae were present at all sites near the maize plantings, but in only one of the six remote sites. In addition, a comparison of the size of an adult showed that mosquitoes breeding near maize plantings were larger.

Implications: This study points to a possible causal relationship between the intensity of malaria transmission and maize culture in Ethiopia and other regions of sub-Saharan Africa. Since maize pollen is an important source of nutrition for the mosquito larvae and maize grows close to where people live, this suggests a potential and previously ignored malaria intervention strategy. While the elimination of maize is not practical in Africa because of its importance as a food crop, the genetic modification of maize plants to alter pollen production may provide a potentially potent anti-malarial intervention tool.

Yihdego Y, Pollack R, and Spielman A: Enhanced development in nature of larval anopheles arabiensis mosquitoes feeding on maize pollen. American Journal of Tropical Medicine and Hygiene 63: 90-93, 2000.

Longitudinal Follow-up of Neonatal Intensive Care Unit Survivors

Background: Many more neonates are living to infancy and childhood, in part because of the utilization of neonatal intensive care units (NICU). Since many major neonatal illnesses are associated with poor school and academic performance, it is important to separate the potential effects of low birth weight from the other neonatal medical status factors that may occur concurrent with low birth weight.

The sample of 188 children followed up to eight years included 39 who were healthy, full term infants as well as 149 preterm infants recruited from one NICU. They NICU "graduates" were grouped according to nature and extent of problems at discharge from NICU: one group had few clinical problems; another group were clinically ill but without neurologic abnormality; a third group had severe neurologic compromise in the neonatal period; a fourth group was small for gestational age with or without medical problems.

All groups were followed at hospital discharge from NICU, at 18 and at 30 months, as well as at 4 and 8 years. Follow-up included comprehensive assessments. The study had low attrition and researchers were blinded to the neonatal status of the children.

Advance: Change in neurologic classification over time varies as a function of the nature and types of neonatal illness and complications, and these changes affect cognitive and school achievement outcomes. The patterns over the long follow-up indicate that factors other than low birth weight and gestational age alone are important to cognitive and school achievement outcomes. A steady increase in abnormal neurologic status occurred in the two groups of children who were discharged from NICU with major problems.

Implications: Though NICU survival has improved, the incidence of diseases and complications in the neonatal period have remained stable. The present study indicates that neonatal medical status is an important variable affecting cognitive and school performance. A child with negative neurologic findings at 18 months or later may require early intervention services at 18 and 30 months of age, speech or physical therapies at preschool age and additional school resources at school age.

McGrath MM, Sullivan MC, Lester BM, and Oh W: Longitudinal neurologic follow-up in neonatal intensive care unit survivors with various neonatal morbidities: Pediatrics 106: 1397-1405, 2000.

Predicting Left Ventricular Hypertrophy in Young Hypertensive African-American Men

Background: he vast incidence of hypertension means that not every individual with hypertension reasonably can be screened for end-organ damage stemming from the hypertension. Additionally, achieving hypertension control (a relatively normal blood pressure) is not sufficient to determine if end organ damage is occurring or progressing.

While microalbuminuria is a predictor of cardiovascular events in hypertension, if target organ damage such as left ventricular hypertrophy (LVH) and proteinuria is found, more aggressive treatment is recommended by the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure. African-Americans are at particularly high risk for hypertension-related renal failure and left ventricular hypertrophy. A random spot urine test was used to measure albumin/creatinine ratio, as a predictor of left ventricular hypertrophy.

Advance: For African-American subjects, the mean age was 41 years with blood pressure mean readings of 157/107. Echocardiogram evidence of left ventricular hypertrophy was present in 27.5 percent of the subjects. A significant correlation was found between echocardiogram evidence of left ventricular mass and urinary albumin/creatinine ratio. The single void, simple, inexpensive urine albumin/creatinine ratio is a predictor of left ventricular mass in young, inner-city, African-American men with hypertension not on medical therapy. The test is practical and less expensive than other tests which might be used to screen for end organ damage.

Implications: The population of young, African-American, urban men is particularly difficult to recruit into screening and treatment for chronic disease. Echocardiograms are not always available in an outpatient or store-front health care setting. Having a simple, inexpensive test that helps screen patients who already have evidence of organ damage from untreated or inadequately treated hypertension helps target those patients for special effort, since they have the most to gain from aggressive antihypertensive therapy.

Post WS, Blumenthal RS, Weiss JL, Levine DM, Thiemann DR, Gerstenblith G, and Hill MN: Spot urinary albumin-creatinine ratio predicts left ventricular hypertrophy in young hypertensive African-American men. American Journal of Hypertension 13: 1168-1172, 2000.

Mouse Models of Insulin Resistance

Background: Type 2 diabetes is the most common form of the disease, affecting about 90 to 95 percent of the 16 million Americans with diabetes. Insulin, a hormone secreted by beta islet cells of the pancreas, reduces glucose levels by promoting glucose uptake in muscle and fat and suppressing glucose production in the liver. In many individuals, especially those who are obese, muscle, liver and fat cells lose the ability to respond to insulin effectively, and they develop so-called "insulin resistance." The pancreatic beta cells must produce ever-increasing amounts of insulin to maintain normal blood sugar levels. For reasons that are poorly understood, the pancreas is unable to keep up with this increased demand in some individuals, and they progress from insulin resistance to development of diabetes. Obesity is associated with insulin resistance and is a major risk factor for type 2 diabetes. Researchers believe that an increase in fat in genetically susceptible individuals contributes to insulin resistance in muscle and liver.

Advance: To gain insights into the connection between obesity and type 2 diabetes, investigators generated mice lacking GLUT4 in fat. This protein mediates insulin-stimulated glucose uptake in fat and muscle. Although GLUT4 was only absent in fat, both muscle and liver cells demonstrated a decreased ability to respond to insulin. This finding suggests that fat cells normally secrete a factor that travels in the blood to the muscles and liver and that the absence of GLUT4 changes the amount of this factor that is released into the blood. Support for this concept comes from experiments demonstrating that muscle removed from the knockout mice, and therefore removed from control by factors released by the defective fat cells, responds appropriately to insulin. Researchers concluded that fat cells must use messengers to "talk" to liver and muscle cells. These results also demonstrate that glucose transport in fat plays a critical role in maintaining glucose balance. The decreased activity of GLUT4 in fat seen in human obesity and type 2 diabetes may contribute to overall insulin resistance and to the risk of developing type 2 diabetes.

In related research, investigators generated mice with a disruption of the *GLUT4* gene in muscle to determine how muscle tissue develops insulin resistance in diabetes. In this mouse knockout model, insulin was significantly impaired in its ability to stimulate glucose uptake in fat and to suppress glucose production by the liver. When the investigators treated the mice with drugs to prevent the elevation in glucose, they were able to restore normal insulin action in both fat and liver, suggesting that prolonged exposure to elevated blood glucose levels can lead to insulin resistance in these tissues. This is the first evidence that a primary defect in insulin-stimulated glucose uptake in muscle can cause insulin resistance in liver and fat, thereby resulting in development of diabetes.

To examine other pathways that may play a role in insulin resistance, researchers created mice lacking a functional gene for the enzyme Akt2. Akt2 is one of a chain of enzymes in the signaling pathway by which insulin controls glucose uptake into muscle and fat as well as glucose production by the liver. Mice lacking functional Akt2 had elevated blood glucose levels because both the ability of insulin to increase glucose uptake and its ability to decrease glucose

production were impaired. This mouse model mimics human impaired glucose tolerance – a pre-diabetic condition in which the body fails to process glucose efficiently. It also provides evidence that Akt2 is required for insulin to control the liver's glucose production and to stimulate muscle's glucose uptake.

Implications: These advances demonstrate the enormous contributions of knockout mouse models to our understanding of the disease process. By identifying the pathways involved in the development of type 2 diabetes and obesity, these models provide targets for drug discovery and useful models to test potential new drugs. [secondary – prevention]

Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hydro E, Minnemann T, Shulman GI, and Kahn BB: Adipose-selective targeting of the *GLUT4* gene impairs insulin action in muscle and liver. Nature 409: 729-733, 2001.

Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB, Kaestner KH, Bartolomei MS, Shulman GI, and Birnbaum MJ: Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKBβ). Science 292: 1728-1731, 2001.

Kim JK, Zisman A, Fillmore JJ, Peroni OD, Kotani K, Perret P, Zong H, Dong J, Kahn CR, Kahn BB, and Shulman GI: Glucose toxicity and the development of diabetes in mice with muscle-specific inactivation of GLUT4. Journal of Clinical Investigation 108: 153-160, 2001.

Integration of Diverse Signals Regulating Appetite and Body Size by the Brain

Background: Obesity is a growing problem in the United States and Western world. A number of studies indicate that choices about food intake are not all made at a conscious level. The regulation of appetite – and the control of body weight – is under the influence of a highly complex web of molecular signaling molecules through which a range of diverse tissues and organs communicate with one another. In recent years scientists have developed a clearer picture of a surprisingly intricate system within the brain that integrates these signals and determines not just when but also what and how much we eat.

In order for a hormone to exert an effect on a target cell, that cell must express a protein, termed a receptor, that detects the presence of the hormone and transduces its signal. Cells that lack such receptors are not directly affected by the hormone because they do not "see" it. The presence of a receptor for a particular hormone therefore identifies that cell as a potential target of that signaling molecule. Researchers can use information about the distribution of receptors among cells in various tissues to identify not only where hormones are likely to act, but by inference, their functions. The human brain contains many billions of cells with a maze of complex interconnections. By using hormones and their receptors as tools, scientists have been able to identify highly organized groups of cells in the brain that can integrate signals coming from our stomachs, our senses (smell, taste), even our moods, with hormonal signals from various tissues in the body, all to make us eat, or stop eating.

Advance: Researchers have demonstrated that deletion of the M3 muscarinic receptor, a receptor for one of the major neurotransmitters in the brain, acetylcholine, produces mice who eat less and as a result are leaner than normal mice. In these animals, levels of melanin-concentrating hormone (MCH), a brain neuropeptide that appears to be involved in stimulating eating and which is usually elevated in the fasting state, are much lower than normal. Consistent with this finding, mice genetically modified to over produce MCH are obese and insulin-resistant. Both acetylcholine and MCH are distributed widely throughout the brain and provide interconnections to the small region of the brain, the hypothalamus, which directly controls eating. This area of the brain also contains receptors for many hormones from other parts of the body, such as insulin and leptin, that signal the status of the body's energy reserves. Mice who lack insulin receptors in the brain eat more than normal mice, become obese, and show signs of type 2 diabetes, including insulin resistance and high blood sugar. Mice who have either spontaneous or genetically engineered mutations blocking leptin receptor function are also obese. Leptin receptors are found in only a few cells in the brain and scientists have used this as a tool to study how leptin and other signals might be translated into reduced eating behavior. These cells contain a variety of neuropeptides, some of which have been shown to inhibit eating and some which have been shown to stimulate eating. By using a variety of powerful and highly sophisticated techniques, researchers were able to demonstrate how leptin acts to inhibit eating. Leptin acts directly on cells that activate brain pathways which stimulate eating behavior and suppresses their function. At the same time, leptin acts directly on cells that activate satiety centers, those parts of the brain that tell us we are full, to enhance their activity. Surprisingly, these cells work not only by inhibiting food intake directly, but they also send signals to the cells

which are inhibited by leptin and block them even further. Brain insulin receptors are also localized to this same small brain region, providing further support to its central role in the integration of the diverse array of signals that control our eating behavior. All of this data indicates a high degree of cross-talk and communication amongst a variety of signaling pathways and suggests that these pathways are also linked to higher cortical centers within the brain.

Implications: These results describe a rich and complex system in which signals generated by a range of tissues converge at the brain and are synthesized into a coherent message that ultimately may or may not prompt a person to eat. A perturbation in just one aspect of this network has the potential to disrupt this delicate balance and, in doing so, could lay the groundwork for increased food intake which, if unchecked, could lead to obesity and its complications. Understanding the nature of these diverse signals, how they interact with one another, and how their messages are reconciled with one another by the brain may not only reveal new insights about the complex nature of appetite regulation but also open new avenues for possible treatments of this growing problem. [Knowledge: Endocrinology/Metabolism/Musculoskeletal/Dermatology/ Gastrointestinal]

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Yamada M, Miyakawa T, Duttaroy A, Yamanaka A, Moriguchi T, Makita R, Ogawa M, Chou C, Xia B, Crawley JN, Felder CC, Deng C-X, and Wess J: Mice lacking the M3 muscarinic acetylcholine receptor are hypophagic and lean. Nature 410: 207-12, 2001.

Increasing Severity of Diabetes in Younger Native Americans

Background: Diabetes is a major health problem for Native Americans. In some communities, almost half of all adults suffer from diabetes. Epidemiologic data indicate that the incidence of obesity-induced type 2 diabetes is dramatically rising among Native Americans, and it is increasingly being diagnosed at younger ages. Diabetes is characterized by high levels of blood sugar, and uncontrolled blood sugar leads to severe complications. The amount of hemoglobin A1c in a patient's blood is a surrogate marker of blood sugar levels and can be used to estimate the average blood sugar over the previous 8-12 weeks. High levels of hemoglobin A1c indicate that blood sugar is not well controlled and are a very accurate predictor of future diabetic complications, including blindness, kidney disease, cardiovascular disease, and premature death.

Advance: Researchers examined dietary information and hemoglobin A1c data collected from diabetic members of the Pueblo Indian tribes in New Mexico. They found that the youngest patients are those with the highest levels of hemoglobin A1c, indicating poorly controlled blood sugar. Blood sugar control can be affected by diet and body weight. Even after adjusting for differences among the age groups in body weight, treatment, diet and physical activity, those at younger ages had higher levels of hemoglobin A1C. In dietary surveys, the youngest diabetes patients indicated that they ate, on average, more high-fat and high-sugar foods than did older diabetes patients. Thus, the youngest patients have the highest rates of poorly controlled blood sugar and eat foods likely to compound their problems by interfering with blood sugar control and increasing body weight.

Implications: Because poorly controlled blood sugar and duration of diabetes are accurate predictors of future diabetic complications, these data predict that the youngest diabetic Pueblos will suffer from the most severe complications if their blood sugar remains poorly controlled. Moreover these complications will occur at younger ages, resulting in greater loss of life and function. This study emphasizes the drastic need to improve blood sugar control among young diabetic Pueblos. Because young patients report eating a diet high in fat and sugar, promoting a return to traditional Native American diets, which are low in animal fat and sugar, could improve control of blood sugar and reduce complications. In addition, young, Native Americans with diabetes need to be much more vigorously treated to achieve recommended levels of glucose control and prevent future complications.

Carter JS, Gilliland SS, Perez GE, Skipper B, and Gilliland FD: Public health and clinical implications of high hemoglobin A_{1c} levels and weight in younger adult Native American people with diabetes. Archives of Internal Medicine 160: 3471-3476, 2000.

Improved Long-Term Survival for Patients with Type 1 Diabetes

Background: Before the introduction of insulin as a treatment for diabetes in the 1920's, the onset of type 1 diabetes – usually in childhood – meant almost certain death. A hospital-based study of Americans has shown that people with type 1 diabetes diagnosed between 1950 and 1981 had mortalities up to seven times higher than the general population. Very limited population-based information is available regarding time trends in mortality of diabetes for patients diagnosed in recent years. This information could yield important insights about possible improvements in outcomes for people with diabetes because medical care has improved greatly with the introduction of self-monitoring blood glucose, the measurement of hemoglobin A1C, and better blood pressure management.

Advance: Researchers examined the mortality rate within the Allegheny County (Pennsylvania) Registry of patients, a group of individuals whose type 1 diabetes was diagnosed before their 18th birthdays between 1965 and 1979. The patients who comprise this group have been living with diabetes for an average of over 25 years. In order to determine whether recent technological and therapeutic advances have had an impact on the long-term survival of people with type 1 diabetes, the researchers divided this population into three groups based upon time of diagnosis – those diagnosed between 1965 and 1969, between 1970 and 1974, and between 1975 and 1979. The researchers found clear evidence that survival rates for the three cohorts of patients had improved over time. For example, death rates between 10 and 20 years after diagnosis of type 1 diabetes fell from 8.4 percent in the earliest group to 3.5 percent in the latest group. These results suggest that new treatments had a measurable impact on the survival of the patients diagnosed in more recent years. Improved survival was evident in both male and female patients. Both African-Americans and Caucasians demonstrated improvement in outcomes, although mortality remained substantially higher in African-Americans.

Implications: A major improvement in long-term survival was observed in patients diagnosed with type 1 diabetes in more recent years. This improvement roughly corresponds with the introduction of more advanced methods of assessing glucose control and self-monitoring equipment and an improvement in blood pressure therapy in the 1980's. Continued follow-up will be necessary to document whether survival continues to improve over time, to identify reversible causes of mortality and to suggest strategies to further reduce mortality associated with this disease, and particularly the racial disparities in survival.

Nishimura R, LaPortre RE, Dorman JS, Tajima N, Becker D, and Orchard TJ: Mortality trends in type 1 diabetes. Diabetes Care 24: 823-828, 2001.

Cholesterol Transport in Niemann-Pick Type C Disease

Background: Niemann-Pick Type C (NP-C) is a rare neurological disorder, affecting an estimated 500 children in the U.S. The disease is inherited when a child receives two mutant genes, one from each parent. In NP-C, cholesterol derived from low-density lipoprotein, commonly known as "bad cholesterol," accumulates in cells of the brain, liver, spleen, lungs, and bone marrow. This cholesterol build-up leads to an enlarged spleen and liver, poor muscle control, impaired eye movements, slurred speech, and dementia. NP-C is always fatal, usually by age 15, and there is currently no proven treatment to slow the course of the disease. The key defect of NP-C appears to lie in cholesterol transport within the cell.

Advance: Previously, researchers identified one of the genes, *NPC1*, which is responsible for 95 percent of NP-C cases. Now, the function of the normal NPC1 protein has been determined. It was expected that the NPC1 protein, located in lysosomal compartments within the cell, would play a key role in cholesterol transport. Instead, investigators determined that the NPC1 protein resembles a family of bacterial permeases that transport various substances throughout the bacterial cell membrane. Researchers now believe that NPC1 is a permease that can transport lipids such as fatty acids, but apparently not cholesterol, across membranes.

In related work, researchers identified a second gene, *HE1*, responsible for the remainder of NP-C cases. Disease caused by mutations in *HE1* is sometimes called NPC-2. *HE1* encodes for a cholesterol-binding protein found in many cell types throughout the body. *HE1* was found in normal skin cells but not in skin cells from NPC-2 patients. In addition, researchers found that adding normal HE1 protein to cells derived from NPC-2 patients restored the ability of the abnormal cells to transport cholesterol. Gene sequencing further confirmed that the *HE1* gene from NPC-2 patients contained mutations that made it unable to function. Unaffected individuals or those with mutations in *NPC-1* had no mutations in *HE1*.

Implications: Regardless of which gene is defective, NP-C patients have a similar clinical and biochemical profile suggesting that *NPC1* and *HE1* may interact or function together in a common metabolic pathway. The identification of *HE1* and the determination of the function of NPC1 should further the understanding of how cells normally handle cholesterol and its transport within the cell. This new knowledge could eventually lead to new treatments for NP-C patients, as well as for the much larger population with elevated blood cholesterol levels.

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Naureckiene S, Sleat DE, Lackland H, Fensom A, Vanier MT, Wattiaux R, Jadot M, and Lobel P: Identification of *HE1* as the second gene of Niemann-Pick C disease. Science 290: 2298-2301, 2000.

Beta Cells that are Resistant to Immune-mediated Destruction

Background: Under normal conditions, the immune system protects the body from potentially harmful invaders when a threat is detected. In type 1 diabetes, insulin-producing beta cells in the pancreas are misidentified as "foreign" and destroyed. The immune response involves a range of defenses, including the mobilization of T cells, a specialized cell type that is "activated" by foreign molecules and proliferates rapidly to mount a counter-attack against the invader. Beta cells may be particularly vulnerable to attack by the immune system as their ability to withstand and repair damage seems to be lower than that of other cell types. Discovering ways to increase the ability of beta cells to withstand such an assault could be important in prevention.

Advance: To gain insight into the role of the immune system in the development of type 1 diabetes, scientists studied two strains of mice: the non-obese diabetic (NOD) mouse and the ALR/Lt mouse, which share a common origin. Despite a very high degree of genetic relatedness, the NOD mouse is highly susceptible to type 1 diabetes while the ALR-Lt mouse is resistant. To better understand why this is so, scientists tested the ability of beta cells of ALR/Lt mice to withstand manipulations that destroy these cells and lead to diabetes in NOD mice. They found that isolated beta cells taken from ALR/LT mice did not experience negative effects from exposure to factors that, in NOD mice, are known to promote inflammation and destroy the insulin-producing capacity of beta cells. Incubation of islets with activated T cells produced similar results. To see if beta cells in the ALR/Lt mouse were somehow resistant to immune-mediated destruction, the researchers irradiated NOD and ALR/Lt mice – destroying their bone marrow – and then infused NOD bone marrow into both sets of animals. After 12 weeks, 80 percent of NOD mice developed diabetes and by 18 weeks 100 percent had. In contrast, no ALR/Lt mice developed diabetes during this time, indicating that the beta cells in these mice had not been destroyed by NOD-derived immune cells.

Implications: Much prior research on type 1 diabetes has focused on the actions of the immune system. Recent methodological advances in the identification and profiling of activated T cells, including an assay that enriches the most active T cells, promise to further advance attempts to design immune-based therapies. Far from being passive victims of the immune system, the beta cells in ALR/Lt mice seem to actively resist destruction by both chemical and biological means. Furthermore, when ALR/Lt mice are mated with NOD mice, the offspring are also resistant to diabetes, suggesting that the resistance of ALR/Lt mice to diabetes is a dominant characteristic. The genetic basis for this trait is unknown, but its ultimate discovery could have important clinical implications, particularly if it could be turned on or enhanced in individuals at risk for type 1 diabetes. [secondary – instruments]

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Novak EJ, Masewicz SA, Liu AW, Lernmark Å, Kwok WW, and Nepom, GT: Activated human epitope-specific T cells identified by class II tetramers reside within a CD 4^{high}, proliferating subset. International Immunology 13: 799-806, 2001.

Molecular Mechanisms of Cellular Copper Metabolism

Background: Copper is an essential element for growth and development in all living organisms. It is required for cellular respiration, iron balance, pigment and connective tissue formation, neurotransmitter production, protein building, and antioxidant defense. The inherited disorders of copper transport, Menkes and Wilson disease, dramatically highlight both the necessity for copper and the danger of toxicity when copper exceeds cellular needs. This balance is achieved at the cellular level as well as in tissues and organs. A fatal defect in a gene in Menkes patients leads to excess storage of copper in the intestine and kidney causing copper deficiency in the remaining parts of the body. In Wilson's disease, a defect in the gene whose expression is liver-specific leads to a toxic build-up of copper in the liver and, eventually, in the brain. These two diseases are caused by mutations in distinct, but nearly identical genes, encoding copper-transporting enzymes that enable cellular egress of copper. It is the genetic nature of these disorders that has provided an opportunity to clarify aspects of intact cellular transport systems that regulate uptake and export of copper.

Advance: Proper functioning of transport mechanisms controlling uptake and egress of copper is essential to maintaining perinatal copper stability in Menkes and Wilson disease. Researchers have recently identified a family of proteins, known as metallochaperones, that are believed to play a role in intracellular copper trafficking. The delivery of copper to intracellular targets in mammals is mediated by the metallochaperone Atox1. To clarify the specific function of Atox1 in mammalian cells, and its role in cellular copper stability, investigators engineered a genetic mouse model with a disruption of the *Atox1* locus. These mice failed to thrive immediately after birth. Those that did survive exhibited growth failure, flacid skin, and seizures due to copper deficiency. Maternal Atoxl deficiency increased the severity of the physical traits presented. In addition, Atox1-deficient cells stored high levels of copper, a defect attributed to impaired egress of cellular copper. In studies of a different human gene, *CTR1*, that was suggested to encode a protein important in copper uptake (based on its similarity to yeast proteins), researchers generated and analyzed mice carrying a gene for *CTR1* that had been inactivated. They observed deadly effects in homozygous mutant embryos and a deficiency in copper uptake in the brains of heterozygous animals. These results showed that CTR1 is essential for embryonic growth and development and necessary for copper transport into the brain.

Implications: Characterization of the molecular genetic basis of the inherited disorders of copper transport seen in Menkes and Wilson disease reveals a direct role for the metallochaperone Atox1 in trafficking of intracellular copper to the secretory pathway of mammalian cells, thus demonstrating its critical role in perinatal copper stability. Evidence for a required role of CTR1 in mammalian copper uptake in the developing and adult animal also implicates CTR1 in embryonic development and as the needed port of copper entry into the brain.

Hamza I, Faisst A, Prohaska J, Chen J, Gruss P, and Gitlin JD: The metallochaperone Atox1 plays a critical role in perinatal copper homeostasis. Proceedings of the National Academy of Sciences USA 98: 6848-6852, 2001.

Kuo YM, Zhou B, Cosco D, and Gitschier J: The copper transporter CTR1 provides an essential function in mammalian embryonic development. Proceedings of the National Academy of Sciences USA 98: 6836-6841, 2001.

Effect of the Protease Inhibitor Indinavir on Import of Glucose into Muscle

Background: Current therapy for HIV infection usually involves a multi-drug regimen known as highly active antiretroviral therapy (HAART), which includes an HIV protease inhibitor (PI). Prolonged HAART is associated with a potentially serious metabolic syndrome that may result in the redistribution of body fat from the extremities to the trunk, lipid abnormalities, and insulin resistance or diabetes. The cause of this syndrome is unknown, but it is thought to be a complex physiological response to one or more of the components of the antiretroviral drug regimen.

Advance: The import of glucose from the blood by skeletal muscle is an important component of normal metabolism and is critical for the maintenance of normal levels of blood glucose. In order to determine the impact, if any, of PI therapy on this process, scientists studied whole muscles isolated from rats. By incubating the muscles in a solution containing radioactive glucose, the researchers were able to monitor the rate at which the sugar was imported into the muscle. As expected, the addition of insulin dramatically increased the amount of glucose imported into the muscle. When the PI indinavir was added as well, glucose import was decreased by 40 to 58 percent. Similar results were observed when glucose import was promoted by contraction of the muscle. A close examination of the molecular basis for these changes revealed that indinavir did not impair the ability of insulin to signal the cell to import glucose; rather, it inhibited the transport of the glucose molecules across the cell membrane. Further evidence for a role of indinavir in glucose metabolism comes from a study of ten HIV-negative men who were given the drug for four weeks to determine its effect on metabolism in healthy individuals. Indinavir therapy resulted in significant increases in fasting blood glucose levels, higher insulin levels, higher insulin-to-glucose ratios, and increased insulin resistance. All of these findings indicate an impairment in normal glucose metabolism.

Implications: The cause of the HAART-associated metabolic syndrome is unknown. These studies indicate that, even in healthy individuals, the PI indinavir can significantly impair glucose metabolism, suggesting that PIs may contribute to the metabolic changes seen in patients receiving HAART. This seems to be a result – at least in part – of impaired import of glucose in skeletal muscle. These studies offer important insights into the understanding of the physiological causes of HAART-associated metabolic complications and may lead to the refinement of HIV therapeutic approaches.

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Noor MA, Lo JC, Mulligan K, Schwarz JM, Halvorsen RA, Schambelan M, and Grunfeld C: Metabolic effects of indinavir in healthy HIV-seronegative men. AIDS 15: F11-F18, 2001.

Helicobacter pylori Strains Influence Host Inflammatory Responses

Background: While it is well established that the bacterium *Helicobacter pylori* increases the risk for ulcer disease and gastric cancer, the majority of *H. pylori* colonized patients remain asymptomatic. The likelihood that individuals will develop disease may depend on characteristics of the host, bacterial factors, or the interactions between host and microbe. The mechanisms have not been clearly described by which certain *H. pylori* strains become associated with disease outcomes. One constituent of an *H. pylori* strain specifically related to an enhanced risk for ulcer disease and distal gastric cancer is the *cag* pathogenicity island. The *cagA* strains appear to be disproportionately represented among persons who develop serious sequelae of *H. pylori* infection. Recently, it has been shown that one of the proteins produced by the pathogenicity island, CagA, is transported into the epithelial cell, where it may participate in cell activation and contribute to disease caused by other *cag* island genes. While research in *H. pylori* pathogenesis has used multiple different animal models, recent interest has focused on the gerbil, since, like humans, it may develop gastric cancer during *H. pylori* infection.

Advance: To determine the ability of two *H. pylori* isolates to induce different host responses *in vivo* or *in vitro*, researchers examined gerbil isolates from components of two strains of bacteria that have similar gene profiles as related to virulence, but which induce distinct responses based on genetically expressed characteristics. They then used an *H. pylori* whole genome microarray to analyze expression of many genes at once in order to identify bacterial determinants of developing disease. Their results showed that gastric ulcer strain B128 induced more severe gastritis, proliferation, and cell death in gerbil mucosa than did duodenal ulcer strain G1.1. Gastric ulceration and atrophy occurred only in B128 gerbils. The microarray identified several strain-specific differences in gene makeup, including a large deletion of the *cag* island genes in strain G1.1. Disruption in the *cag* island in strain B128 significantly decreased gastric inflammation *in vivo* and reduced inflammatory mediators *in vitro*.

Implications: With this advance, investigators have shown that *H. pylori* strains having similar virulence markers are able to induce different types of gastric inflammation and injury in an animal model. Furthermore, these findings can be validated *in vitro* and *in vivo*. The *H. pylori* entire genome microarray revealed that distinctions in the ability of *H. pylori* strains to induce epithelial cell responses, which are related to inflammation, are dependent on the presence of an intact cag pathogenicity island. The specific function of additional strain-specific loci and their significance in the development of disease remain to be determined.

Israel DA, Salama N, Arnold CN, Moss SF, Ando T, Wirth HP, Tham KT, Camorlinga M, Blaser MJ, Falkow S, and Peek RM: *Helicobacter pylori* strain-specific differences in genetic content, identified by microarray, influence host inflammatory responses. Journal of Clinical Investigation 107: 611-620, 2001.

What Causes Polycystic Kidney Disease?

Background: The kidneys of patients who inherit Polycystic Kidney Disease (PKD) gradually enlarge and stop working properly. Kidney function is compromised by the growth of fluid-filled cysts that gradually "squeeze out" the normal kidney tissue. Researchers celebrated a breakthrough when they identified two genes that, when mutated, are responsible for causing autosomal dominant polycystic kidney disease (ADPKD). Before this knowledge can be used in developing PKD treatments, however, it is critical to know what these genes normally do within the kidney.

Advance: Scientists have recently described the normal function of the genes that cause ADPKD when they are mutated. The PKD-1 and PKD-2 genes give rise to proteins, called polycystin-1 and polycystin-2, respectively. Experiments suggest that polycystin-1 and -2 interact to form a channel, or opening, on the outer surface of cells that permits passage of calcium and other positively-charged molecules. In kidney epithelial cells, entry of calcium is thought to set off a chain of signals that controls cell growth and promotes normal structure and function of the kidney's filtering tubules. Scientists have identified mutant versions of the polycystin-1 and -2 proteins in patients with ADPKD. One type of mutation prevents polycystin proteins from reaching the cell surface, so they are unable to form a channel. Without the channel, calcium is not able to enter the cells and signaling is disrupted. Disrupted signaling prevents normal maintenance of epithelial cell growth, and may result in generation of fluid-filled cysts. Another study suggests a second possible mutation that could result in signaling disruption. The end of the polycystin-1 protein that is located on the outside of kidney epithelial cells is called the C-terminus. The C-terminus of polycystin-1 must be intact in order for calcium and other positively-charged molecules to enter kidney epithelial cells. A mutation in PKD-1 that causes loss of the polycystin-1 protein C-terminus would prevent entry of calcium and other positively-charged molecules into the cells, again causing signaling disruption and possibly resulting in cyst formation.

Implications: These studies have increased our understanding of how the proteins made from the PKD-1 and PKD-2 genes work. Because scientists now have a better understanding of how the polycystin proteins function in normal kidneys, they can design better ways to counteract or overcome the mutated polycystin proteins inherited by ADPKD patients. Thus, the vital information provided by these studies could lead to the development of new and improved approaches for treating polycystic kidney disease.

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Vandorpe DH, Chernova MN, Jiang L, Sellin LK, Wilhelm S, Stuart-Tilley AK, Walz G, and Alper SL: The cytoplasmic C-terminal fragment of polycystin-1 regulates a Ca^{2+}-permeable cation channel. Journal of Biological Chemistry 276: 4093-4101, 2001.

Advances in Understanding the Function, Structure, and Genetics of the Urinary Bladder

Background: Researchers are discovering that the urinary bladder is much more than a reservoir for liquid waste. Rather, it is a dynamic organ with many important structural and physiological properties. For example, the uroplakins are a class of four proteins that have been shown to be major differentiation products in the mouse, rabbit, bovine, dog, and human bladder lining (urothelium). They are vital in the permeability barrier that protects the bladder from infectious agents, and may provide the key to understanding the basic disease processes in urinary tract infection, interstitial cystitis, and bladder cancer. Plaques of crystalline uroplakin particles almost entirely cover the bladder lining.

Advance: In examining the interactions of the four uroplakin proteins in animals, investigators found that two of the proteins link specifically to the other two (uroplakin UPIa with UPII, and uroplakin UPIb with UPIII). In normal circumstances, the two pairs are present in all plaques, and all plaques have a similar uroplakin composition. They also found that the bladder can secrete a host of different proteins, which may play physiological or pathological roles. They are studying where in the plaque-covered bladder lining the proteins are secreted.

The same group of scientists also report that the removal of the gene for uroplakin III causes defects in the permeability barrier function of the bladder lining, which can lead to a host of abnormalities in the urinary tract. It appears that both uroplakin pairs are required for normal plaque formation. Using a mouse model, researchers have shown that knocking out the UPIII gene causes the mouse bladder to become permeable and the ureteral orifice to become wide open, resulting in vesicoureteral reflux. This defect also causes several abnormalities in UPIII's partner, UPIb. The result is that only small patches of urothelial plaque are formed. In humans, vesicoureteral reflux is a hereditary condition that affects about one percent of pregnancies and represents a leading cause of renal failure in infants. Until now, there was no known genetic basis for this disease. These new data now suggest that the absence of uroplakin III can cause this defect. However, the researchers stress that reflux is likely caused by more than one gene.

Implications: Taken with previous knowledge that plaques are dynamic structures that are capable of breaking and fusing, this new information will lead to better understanding of normal and abnormal plaque formation and function and, in turn, of the normal and abnormal function of the urinary bladder. Understanding the genetic and structural bases for vesicoureteral reflux could pave the way to earlier detection and treatment of this disease.

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Hu P, Deng FM, Liang FX, Hu CM, Auerbach AB, Shapiro E, Wu XR, Kachar B, and Sun TT: Ablation of uroplakin III gene results in small urothelial plaques, urothelial leakage, and vesicoureteral reflux. Journal of Cell Biology 151: 961-971, 2000.

Liang FX, Riedel I, Deng FM, Zhou G, Xu C, Wu XR, Kong XP, Moll R, and Sun TT: Organization of uroplakin subunits: transmembrane topology, pair formation and plaque composition. Biochemical Journal 355: 13-18, 2001.

Genetic Link Discovered for IgA Nephropathy

Background: End-stage kidney failure is a major health problem in the U.S., affecting approximately 400,000 people. Glomerulonephritis, a principal cause of end-stage kidney failure, is characterized by inflammation of the tiny capillary bundles in the kidney that serve as filters, separating wastes and extra fluid from the blood. IgA nephropathy is the most common form of glomerulonephritis. It occurs when immunoglobulin A (IgA) forms deposits in the kidney, where it creates inflammation. IgA nephropathy is an autoimmune disease, and researchers are trying to discover why these deposits are formed and how formation might be inhibited.

Advance: IgA nephropathy is not normally thought of as a genetic disease. However, there is ethnic variation in prevalence, and there also is some evidence for familial clustering. Family members of patients with IgA nephropathy also show kidney abnormalities even though they may not have overt signs of the disease. Researchers analyzed genome-wide scans of 30 families – 24 from Italy and six from the U.S. – that included 94 members with IgA nephropathy and 48 unaffected members. They identified a region on chromosome 6 that is very strongly associated with IgA nephropathy. The pattern of inheritance of the IgA linkage is compatible with the hypothesis that patients with IgA nephropathy inherit susceptibility to the disease, for which there are environmental or genetic modifiers.

Implications: These findings open up a new area of genetic research, and suggest that other kidney diseases that are not commonly thought of as simple genetic disorders may, in fact, have susceptibility genes. Investigators will now proceed to try to identify the IgA nephropathy gene. Identification of the gene may provide insights into the disease process, diagnosis, and treatment of IgA nephropathy.

Gharavi AG, Yan Y, Scolari F, Schena FP, Frasca GM, Ghiggeri GM, Cooper K, Amoroso A, Viola BF, Battini G, Caridi G, Canova C, Farhi A, Subramanian V, Nelson-Williams C, Woodford S, Julian BA, Wyatt RJ, and Lifton RP. IgA nephropathy, the most common cause of glomerulonephritis, is linked to 6q22—23. Nature Genetics 26: 354-357, 2000.

HIV-associated Kidney Disease and the Kidney as a Reservoir of Persistent HIV Infection

Background: The question of what cells in the body serve as reservoirs of HIV virus is important in the development of strategies for long term therapy of AIDS. Human Immunodeficiency Virus type 1 (HIV)-associated kidney disease is the third leading cause of end stage renal disease among African-American men aged 20 to 64. The underlying cause of HIV-associated kidney disease is probably direct viral infection of renal cells, although this has been the subject of controversy. Mice who have been genetically engineered to contain a portion of the HIV virus display changes in their kidneys that are identical to those seen in humans with HIV infection, suggesting HIV proteins in the kidney trigger the development of kidney disease.

Advance: A carefully studied case of HIV renal disease has contributed importantly to our understanding of HIV nephropathy. A 35 year old man was hospitalized with a range of symptoms consistent with HIV. A blood test confirmed that he was HIV-positive. A kidney biopsy showed clear evidence of focal glomerulosclerosis, a type of kidney disease. Analysis of the tissue sample readily demonstrated HIV within the cells of the kidney. The man was treated with highly active antiretroviral therapy for his HIV infection and dialysis for his kidney disease. After three months, his blood HIV levels had dropped significantly and a second kidney biopsy revealed that much of the tissue had reverted to a more normal appearance; however, traces of HIV were still present within the kidney cells. This observation suggests that, while highly effective therapy can reduce HIV levels below the threshold to cause kidney disease, minute quantities of the virus remain in the kidney. The persistence of HIV in the face of effective therapy has been noted previously in peripheral blood lymphocytes, but has not been seen before in kidney cells.

Implications: The continued presence of HIV within the kidney, even in the face of successful antiviral therapy, suggests that the kidney may represent a long-term reservoir of HIV. Cessation or interruption of therapy could therefore lead to return of disease.

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Surprisingly Broad Potential Fates for Adult Stem Cells

Background: The identification of stem cells in adult tissue – and the discovery of ways to induce them to differentiate – would provide a vital resource that could be used to repair damaged tissue and organs. Such targeted repair would eliminate the need for life-long pharmacological suppression of the immune system currently necessary after an organ transplant. It had previously been thought that adult stem cells were relatively limited in the range of tissues into which they could differentiate. Recently, however, evidence has accumulated to suggest that these cells may have a much wider range of tissues into which they can differentiate; that is, they display greater "plasticity" than previously believed.

Advance: Hematopoietic stem cells – which are derived from bone marrow – represent a plentiful, relatively well-characterized type of adult stem cell. Hematopoietic stem cells possess the twin abilities to self-renew while remaining in an undifferentiated state and to differentiate into a variety of mature cell types, including both red and white blood cells. Many studies have sought to determine whether hematopoietic stem cells can be coaxed to differentiate into cells beyond those of the blood lineage.

Researchers studying a mouse model of hereditary tyrosinemia type I, a fatal liver disease, have successfully used highly purified hematopoietic stem cells to restore normal liver function in half of the animals treated. In similar studies, another group of scientists found that adult mouse pancreatic cells could also restore liver function – albeit in only about ten percent of cases.

Perhaps the most vivid demonstration of the potential plasticity of adult hematopoietic stem cells comes from a study in which female mice – whose natural bone marrow had been destroyed – received a single purified hematopoietic stem cell from a male donor mouse. If the cell infused were not capable of repopulating the mouse's natural bone marrow and differentiating into all cells of the blood lineage, the recipient mouse would not survive. After nearly a year, five female mice survived this experiment. When the tissues of these animals were examined, a significant fraction of the blood cells in the females were found to possess Y chromosomes and therefore to be derived – as expected – from the donated male bone marrow. When the scientists examined other tissues, the result was quite surprising: cells containing Y chromosomes were found in the liver, lungs, gastrointestinal tract, and skin. A single stem cell was therefore able to differentiate not only into cells that could repopulate the animals' depleted bone marrow, but also into cells that became integrated into a number of other organs. The scientists speculated that the stem cells have been drawn to these tissues in order to repair damage caused by the initial irradiation. Additional support for the ability of stem cells to "target" damaged tissue for repair comes from studies in mice that show hematopoietic stem cells can repair injured heart muscle following an experimentally-induced heart attack.

Implications: A previously undescribed population of stem cells with a great deal of plasticity may exist within the bone marrow of adult mice. These stem cells seem to be capable both of reconstituting a functional hematopoietic system – including progenitor and mature blood cells – as well as repairing defects and injuries across a range of tissues, including liver and cardiac

muscle. Additional work indicates that another population of stem cells capable of forming liver tissue – albeit somewhat less efficiently – may be present within the pancreas. Together, these findings suggest a richness and diversity of potential fates for adult stem cells heretofore unappreciated.

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Hematopoietic Differentiation of Rhesus Monkey Embryonic Stem Cells in Culture

Background: Pluripotent embryonic stem (ES) cells have been derived recently from the rhesus monkey. These cells are potentially capable of differentiating to form progeny cells characteristic of the various tissues and organs of the body. Furthermore, these monkey cells have properties very similar to human ES cells and may thus serve as very useful models for various aspects of early differentiation of specific human cell types. However, the specific factors that can cause the monkey ES cells to form tissue-specific cell types are largely unknown. Methods to efficiently induce the formation in culture of specific cell types derived from the ES cells are necessary to fully realize the potential of these cells for investigating various aspects of development, both in monkeys and humans.

Advance: These investigators have developed a culture system whereby rhesus monkey ES cells are induced to form hematopoietic cells in culture. Importantly, they have demonstrated that bone morphogenetic protein 4 (BMP-4) is a crucial factor for promoting robust hematopoietic development in this system. In the presence of BMP-4, cells characteristic of lineages that ultimately produce red blood cells, white blood cells, and platelets are all produced in the culture system.

Implications: This is the first demonstration that rhesus monkey ES cells have the potential to develop into various types of hematopoietic cells in culture. These ES cells can be used to examine factors that cause differentiation along certain pathways to form specific types of cells.

Li F, Lu S, Vida L, Thomson JA, and Honig GR: Bone morphogenetic protein 4 induces efficient hematopoietic differentiation of rhesus monkey embryonic stem cells in vitro. Blood 98: 335-342, 2001.

Mouse and Rat Genome Sequences: Tools for Understanding the Human Genome

Background: With the working draft of the human genome sequence in hand, scientists now seek to interpret its meaning. The genome sequences of the rat and mouse provide important new genomic tools to uncover the location, structure, and function of human genes. The genomes of these two rodents are about the same size as that of the human (approximately 3.1 billion base pairs) and humans share virtually the same overall set of genes with the mouse and rat. The gene sequences in mice, rat, and humans are shared to a high degree (85 percent sequence identity). By comparing the human genome sequence with those of rat and mouse, similar regions are readily apparent and immediately identify protein coding regions and regulatory sequences. In addition, the genome sequences of the mouse and rat will improve the utility of these model systems to study and understand human disease, and to develop and test new treatments in ways not possible with humans.

Advance: Over the last year, public and private sector entities have joined forces to produce draft genome sequences of the rat and mouse. The Mouse Sequencing Consortium (MSC) – comprising three private companies, six Institutes of the NIH and the Wellcome Trust - was formed in October 2000 to work collaboratively to produce a draft sequence of the mouse genome in six months. In May 2001, the MSC announced that it had achieved its goal of 95 percent coverage of the sequence. As with human genome sequencing, the draft mouse genome sequence will be brought to high quality, final form no later than 2005.

In February 2001, the NIH launched the program to sequence the genome of the laboratory rat. Baylor College of Medicine, Celera Genomics and Genome Therapeutics Corp. are collaborating on whole genome shotgun sequencing of rat DNA. In addition, Baylor will contribute the sequence of individual mapped BAC clones, and the draft genome sequence will be assembled by combining the several types of data.

The sequencing strategy for both mouse and rat combines elements of the map-based and whole-genome-shotgun approaches. This dual strategy takes advantage of the lessons learned during the initial sequencing of the human genome by the International Human Genome Sequencing Consortium and, separately, by Celera Genomics. Both the mouse and rat projects have continued the Genome Project's practice of making sequence data rapidly and freely available to the scientific community.

Implications: Cross species comparisons of genome sequences will reveal a wealth of information about the human genetic code and enhance the utility of mouse and rat models of human disease. [secondary – diagnosis]

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Technique Can Distinguish Hereditary from Non-Hereditary Tumors: May Lead To New Diagnostic Tests for Breast Cancer

Background: In the mid 1990s, scientists identified mutations in the BRCA1 and BRCA2 genes that are the major cause of hereditary breast cancer. Women inheriting these mutations have a 40 to 85 percent lifetime risk of developing breast cancer, as well as an increased risk of ovarian cancer. Differentiating between hereditary and non-hereditary breast cancer tumor types is not easy with traditional techniques. When these tumors are viewed under a microscope, it is very difficult to tell which tumors are caused by BRCA1, BRCA2 and non-inherited mutations Earlier insights regarding BRCA1 and BRCA2 tended to give a fairly static view of the genetic changes that produced a tumor. Newly developed DNA microarray technology offered the opportunity to study the expression of many genes within a tumor cell's metabolic pathways.

A DNA microarray is merely a glass slide with tiny dots of DNA from different genes arranged in a grid-like array. Using microarrays, the activity of thousands of genes can be quickly tested at one time.

Advance: NIH scientists used DNA microarrays to simultaneously assess the activity of 6,000 genes within breast cancer cells and generate a unique gene-expression profile of the breast tumors. The research team examined samples of tumors from 22 breast cancer patients. Seven had mutations in BRCA1; eight had mutations in BRCA2, and the remainder had sporadic cases of breast cancer with no family history of the disease. When the team examined the gene-expression profiles using microarrays, they were able to quickly and accurately differentiate the tumors arising in individuals with BRCA1 and BRCA2 mutations from the sporadic cases. BRCA1- and BRCA2-inherited changes as well as the non-inherited genetic changes were identified. The clear differences in the patterns of gene activity in breast tumors are as unique as a fingerprint, pinpointing into which group a woman's cancer belongs. These fingerprints also revealed key genes involved in tumor development and progression.

This new approach should make it possible for physicians to quickly and accurately diagnose the cause of an individual woman's disease and may ultimately guide decisions about the most effective treatment.

Implications: DNA microarray technology gives a snapshot of exactly which genes are active in a tumor cell. Over the last few decades, scientists have made important progress in understanding the molecular origins of cancer by studying one gene at a time. Now they can look at thousands, and even tens of thousands of genes as they interact to produce a tumor. This capability will have important implications for both diagnosis and treatment. [secondary – diagnosis]

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Advances in Prostate Cancer Research

Background: Prostate cancer is one of the most common cancers in American men, with over 175,000 new cases diagnosed in the U.S. every year. Of all known risk factors for prostate cancer, the most significant is having two or more close relatives (father or brother) with the disease. These men are five times more likely to develop prostate cancer when compared to men with no relatives with the disease. This suggests that susceptibility to prostate cancer involves inherited factors. In recent years, researchers have found several genes that may be involved in hereditary prostate cancer. NIH scientists and their colleagues mapped the first gene, called HPC-1, to human chromosome 1 in 1996. Since then, genome-wide scans have implicated several other genes in this complex disease.

Advances: In the past year alone, NIH researchers and co-investigators have made a number of significant advances in the search for prostate cancer susceptibility genes. Further evidence of multiple genes on chromosome 1 has been confirmed and other studies implicated regions on chromosomes 8 (8p22-23) and 20. A study of affected Finnish families identified a gene responsible for a distinct subgroup of late-onset prostate cancer on the X chromosome (HPC-X).

Gene expression profiles reflect a unique fingerprint of the genes that are active in a cell and provide insight into genes in pathways that play a role in the initiation and/or progression of prostate cancer. These studies have shed light into common biological properties of both familial and sporadic tumors as well as the distinct difference between benign and malignant tumors. Hormonal therapy is the standard treatment for men with metastatic prostate cancer, or cancer that has spread. Often this treatment results in shrinkage of the cancer. Hormone refractory prostate cancer, or prostate cancer that is non responsive to hormone treatment, is a disease that kills approximately 39,000 men per year. Scientists have recently correlated the loss of expression of a specific gene on a chromosome 8p21 with hormone-refractory disease and advanced tumor stage in prostate cancer.

Implications: Availability of a highly detailed working draft of the human genome, along with ongoing studies linking specific chromosomal variations with affected individuals, will help researchers identify the precise DNA sequence of the genes found to be involved in prostate cancer. In addition, gene expression studies, made possible by recent advances in DNA "chip" technology, will further elucidate the underlying biological mechanisms controlling the growth and spread of prostate cancer. Such knowledge will ultimately aid in the development of more accurate methods of predicting those individuals at highest risk, diagnosing prostate cancer, and of tailoring therapies to individuals, based on their unique genetic makeup. [secondary – diagnosis]

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Human Genome Project Develops Multimedia EducationalKit for High School Students and Public

Background: With the sequencing of the human genome and accelerated pace of genetic research, innovative new tools are needed to help the public, both old and young, understand how genomics will improve health and affect our lives.

Advance: The Human Genome Project (HGP) has created a free, limited edition educational multimedia kit to improve life sciences education in the nation's schools by ensuring that science teachers throughout the country have better access to the latest information about the HGP. Designed primarily for high school students in general or introductory biology classes, the kit has also proven useful for college students, voluntary health organizations, and the general public.

The kit, entitled *The Human Genome Project: Exploring our Molecular Selves*, includes a multimedia CD-ROM, video documentary, commemorative wall poster; and informational brochure. Since its release in February 2001, nearly 60,000 free kits have been distributed to high school teachers and the public. In the fall of 2001, the kit will be available on the World Wide Web <www.nhgri.nih.gov/educationkit>.

Awarded a gold medal by the New York Film Festival and the CINE Golden Eagle Award, the video documentary, *The Secret of Our Lives*, traces the development, evolution, and impact of the HGP and genomics research. A visual montage of black-and-white and color film footage, eye-catching graphics, and creative camera angles, the video is a far cry from a typical, staid instructional video. With images ranging from DNA expression microarrays to wriggling roundworms, the video provides a venue for HGP leaders and scientists to explain in conversational dialogue what human genome research is all about. This video documentary is closed-captioned and available in both English and Spanish.

The CD-ROM, winner of the Gold Omni Award for multimedia education, includes 3D computer animation illustrating the basics of molecular biology, an interactive timeline of milestones in genetics, and many other teaching and learning tools. The variety of comprehensive information, together with the "game-like" user interface, makes exploring the CD-ROM fun and intellectually stimulating.

Implications: This highly regarded and successful kit is providing an effective tool to educate high school students and their teachers about basic genomics. It also serves as a general educational tool for the public who according to recent polls knows little about the possible implications of genomic research. As genetics is increasingly integrated into regular medical care, it will be important for the public to have a basic understanding of genomics. This tool kit should help bridge that gap in knowledge and inspire some students to pursue careers in scientific research with a specific emphasis on genomics.

Gene Chips Accurately Diagnose Four Complex Childhood Cancers

Background: "Cancer" is actually a broad term used to characterize over 100 distinct diseases that share the common feature of unregulated cell division. There is a group of childhood cancers known as the small, round blue cell tumors (SBCTs) because of their similar appearance under a microscope. These include neuroblastoma (NB), rhabdomyosarcoma (RMS), non-Hodgkin lymphoma (NHL) and the Ewing family of tumors (EWS). In order to successfully diagnose these diseases, it will be important to identify the features of individual cancer cells – their molecular fingerprint. Accurate diagnosis is essential because the treatment options, response to therapy, and prognoses vary widely depending on the diagnosis.

Biological research is increasingly dependent upon information of the entire genome. Development of high-throughput technologies has dramatically enhanced researchers' ability to explore the molecular basis of disease. DNA chips, or microarrays, enable massively parallel molecular analyses in a miniaturized format. Cancer diagnostics will be improved by DNA microarray readouts of the activity levels of thousands of genes.

Advance: Scientists at NIH and Lund University in Sweden developed a method of genetic fingerprinting that can tell the difference between these closely related types of childhood cancer. The method combines, for the first time, the cutting edge technology of DNA microarrays with a form of artificial intelligence called an artificial neural network. The neural network automatically analyzes the enormous amounts of data produced by the microarray to make a highly accurate diagnosis. The research team started by surveying the expression of over 6,000 genes. The neural network analysis narrowed that number down to a mere 93 unique genes needed to differentiate the four tumor types. And of those, 41 were new genes that might provide important insights into the biology of these cancers.

Implications: The newly developed system could prove valuable for anxious parents and family members of sick children. An accurate diagnosis can be critical for the child's survival. When patients get the right therapy, up to 90 percent of the children with Burkitt (a type of non-Hodgkin) lymphoma recover; about half will survive Ewing's sarcoma and rhabdomyosarcoma, and up to 40 percent will recover from neuroblastoma. Without accurate diagnosis and proper treatment, few children survive.

While it may take some time for this technique to reach clinical practice, it already has stimulated additional research. This technology will ultimately define the complete catalog of all the genes involved in cancer. The next challenge will be to determine which of the gene products will make the best targets for new drug treatments. [secondary – diagnosis]

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A Mouse Model Provides Insights about the Inner-ear Defects in Pendred Syndrome, a Genetic Disorder Associated with Deafness and Goiter

Background: Pendred syndrome is a genetic disorder associated with deafness and goiter. The hearing loss is generally profound and occurs before age three, although occasionally it is later in onset and progressive. The goiter in Pendred syndrome is even more variable in its presentation; it can develop at any age, but may be totally absent in some affected individuals. For over 100 years the molecular basis of Pendred syndrome remained largely unknown until 1997, when NIH scientists identified the defective gene (*PDS*).

The *PDS* gene encodes the pendrin protein, which is expressed in thyroid, inner ear, and kidney. Comparison with other proteins in public databases indicated that pendrin is a member of a large family of anion transporters. Functional studies have shown that it is capable of transporting various anions, including iodide, chloride, and bicarbonate.

The goiter encountered in Pendred syndrome is presumably a consequence of defects in pendrin's iodide-transporting capability. The precise role of pendrin in the inner ear is presently less well defined. Localization studies indicate that pendrin is expressed in several discrete areas of the inner ear, providing evidence implicating the absence of pendrin in the inner ear as the cause of deafness in Pendred syndrome.

Advance: To further investigate the functional role of pendrin and to provide a system for more detailed study of the inner-ear defects that occur in the absence of pendrin, a *Pds*-knockout mouse model was generated. In *PDS*-knockout mice, disruption of the *PDS* gene prevents any pendrin protein from being made. While the mice appear normal at birth, they develop early-onset, profound deafness. The inner-ear abnormalities seen in the mice generally mirror the defects seen in individuals with PDS mutation, and thus provide a valuable model for studying the hearing loss associated with PDS mutations.

Implications: This mouse model provides a system to study the mechanisms leading to hearing loss. The observation that the absence of pendrin leads to a profound, progressive deterioration of inner-ear structures rather than a developmental defect suggests a route for therapeutic intervention, which might delay the onset or progression of deafness. This mouse model should provide a valuable experimental tool for investigating possible therapeutic options.

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Scientists Find a New Tumor Suppressor Gene Involved in Breast, Prostate and Other Cancers

Background: Cancer cells have defects in regulatory circuits that govern normal growth. Scientists have made great strides in recent years in determining the key proteins within cells that dictate these regulatory circuits. Tumor-suppressor genes (TSGs) are one of two broad categories of genes involved in cancer. Each cell has two copies of every gene, including TSGs. A cell can function with a single copy of a TSG, but when both copies of a tumor suppressor gene are inactivated, it is like disabling the brakes on a car. Without the function of such a gene, a tumor keeps growing out of control.

Advance: Scientists at NIH and M.D. Anderson Cancer Center at the University of Texas found a novel tumor suppressor gene on human chromosome 7q31 that appears to be involved in a wide range of cancers. The gene, named ST7 – short for Suppression of Tumorigenicity on chromosome 7, is widely expressed in normal tissues throughout the body and is often disrupted by mutation or deletion in tumors arising from epithelial cells, such as cancers of the breast, prostate, colon, and ovary.

Scientists know of at least 30 tumor suppressor genes and a search of gene databases suggests that there may be another 100 or more, so finding a new one alone is not surprising. But the discovery of ST7 demonstrates a new paradigm in molecular genetics research now that the working draft sequence of the human genome is available in public databases. In the past, the discovery of a new TSG would require a major effort involving numerous scientists, often in several laboratories, working for many years. ST7 was discovered by a single post-doctoral scientist using the new tools provided by the Human Genome Project.

Implications: Understanding the molecular basis of cancer is crucial to the development of a new generation of targeted treatments and preventive strategies. New genomic resources are accelerating these advances and hastening progress in cancer research.

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